# Method 642

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### *1. SCOPE AND APPLICATION*

**1.1** This method covers the determination of biphenyl and o-phenylphenol in municipal and industrial wastewater.

| *Parameter* | *CAS No.* |
|---|---|
| Biphenyl | 92-52-4 |
| o-phenylphenol | 90-43-7 |

**1.2** The estimated detection limits (EDL) for the parameters above are listed in Table 1. The EDLs were calculated from the minimum detectable response being equal to 5 times the background noise using a 2-mL final extract volume of a 1-L sample and an injection volume of 50 µL. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.

**1.3** This is a high-performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second HPLC column that can be used to confirm measurements made with the primary column.

**1.4** This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

### *2. SUMMARY OF METHOD*

**2.1** The fungicides are removed from the sample matrix by extraction with methylene chloride. The extract is dried, exchanged to acetonitrile or methanol, and analyzed by liquid chromatography with ultraviolet (UV) detection.

### *3. INTERFERENCES*

**3.1** Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of liquid chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.

**3.1.1** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

**3.1.2** Glassware must be scrupulously cleaned.[1] Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

**3.2** Matrix interferences may be caused by UV-active contaminants that are coextracted from the samples. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limits listed in Table 1.

## *4. SAFETY*

**4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[2-4] for the information of the analyst.

## *5. APPARATUS AND EQUIPMENT*

**5.1** Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and reagent water. Allow the bottles and cap liners to air dry, then muffle the bottles at 400°C for 1 hour. After cooling, rinse the bottles and cap liners with hexane, seal the bottles, and store in a dust-free environment.

**5.1.1** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

**5.2** Kuderna-Danish (K-D) glassware.

**5.2.1** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent) and two-ball micro (Kontes K-569001-0219 or equivalent).

**5.2.2** Concentrator tube: 10-mL, graduated (Kontes K-570050-l025 or equivalent) with ground-glass stopper.

**5.2.3** Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

**5.3** High-performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.

**5.3.1** Gradient pumping system, constant flow.

**5.3.2** Injector valve (Rheodyne 7125 or equivalent) with 50-µL loop.

**5.3.3** Column 1: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Perkin Elmer HC-ODS Sil-X 10 µ, or equivalent.

**5.3.4** Column 2: 250 mm long by 4.6 mm ID, packed with reverse-phase Dupont Zorbax ODS, 6 to 7 µ, or equivalent.

**5.3.5** Ultraviolet detector, capable of monitoring at 254 nm.

**5.3.6** Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)

**5.4** Chromatographic column: 300 mm long by 10 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).

**5.5** Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.

**5.6** Miscellaneous.

**5.6.1** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

**5.6.2** Separatory funnels: 2-L, 500-mL, and 250-mL, equipped with PTFE stopcocks.

**5.6.3** Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride for two hours.

**5.6.4** Water bath: Heated with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

**5.6.5** Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

## *6. REAGENTS AND CONSUMABLE MATERIALS*

**6.1** Reagents.

**6.1.1** Acetone, acetonitrile, methanol, and methylene chloride: Demonstrated to be free of analytes and interferences.

**6.1.2** Reagent water: Reagent water is defined as a water in which an interferant is not observed at the method detection limit of each parameter of interest. The water is held at 90°C. Store in clean, narrow-mouth bottles with PTFE-lined septa and screw-caps.

**6.1.3** Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.

**6.1.4** HPLC Mobile Phase 1: Add 400 mL of acetonitrile to a 1-L volumetric flask and dilute to volume with reagent water.

**6.1.5** HPLC Mobile Phase 2: Add 500 mL of methanol to a 1-L volumetric flask and dilute to volume with reagent water.

**6.2** Standard stock solutions (1.00 µg/µL): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.

**6.2.1** Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality methanol or acetonitrile, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

**6.2.2** Transfer the stock standards to PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

**6.2.3** Stock standards must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

## *7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE*

**7.1** Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices[5] should be followed, except that the bottle must not be prewashed with sample before collection.

**7.2** The samples must be iced or refrigerated at 4°C from the time of collection until analysis.

**7.3** All samples must be extracted and analyzed as soon as possible after sampling, since preservation studies[6] have shown that these compounds undergo almost complete decomposition within seven days.

## *8. CALIBRATION*

**8.1** Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.

**8.2** Prepare calibration standards at a minimum of three concentration levels of the analytes by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase (40% acetonitrile in water or 50% methanol in water). One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

**8.3** Using injections of 50 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results are used to prepare a calibration curve for the analytes. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

**8.4** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.

**8.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

## *9. QUALITY CONTROL*

**9.1** Monitoring for interferences: Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of the analytes, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

**9.2** Assessing accuracy.

**9.2.1** After every 10 samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

**9.2.1.1** Laboratory control standard concentrate: From stock standards prepared as described in Section 6.2, prepare a laboratory control standard

concentrate that contains the analytes at a concentration of 2 µg/mL in methanol or acetonitrile.

**9.2.1.2** Laboratory control standard: Using a pipette, add 1.0 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water contained in a 1000-mL volumetric flask.

**9.2.1.3** Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery ($P_i$) with the equation:

***Equation 1***

$$P_i = \frac{100 S_i}{T_i}$$

*where*

$S_i$ = *Analytical results from the laboratory control standard, in µg/L*

$T_i$ = *Known concentration of the spike, in µg/L*

**9.2.2** At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.[7]

**9.3** Assessing precision.

**9.3.1** Precision assessments for this method are based upon the analysis of field duplicates (Section. 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of the analytes.

**9.3.2** Calculate the relative range[7] ($RR_i$) with the equation:

***Equation 2***

$$RR_i = \frac{100R_i}{X_i}$$

*where*

$R_i$ = *Absolute difference between the duplicate measurements* $X_1$ *and* $X_2$, *in µg/L*

$X_i$ = *Average concentration found* $\left(\frac{X_1 + X_2}{2}\right)$, *in µg/L*

**9.3.3** Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

## *10. PROCEDURE*

**10.1** Sample extraction.

**10.1.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.

**10.1.2** Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.

**10.1.3** Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

**10.1.4** Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, collecting the extract in a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the Erlenmeyer flask and column with about 30 mL of methylene chloride to complete the transfer.

**10.1.5** Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower

rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. If the extract requires cleanup, proceed to Section 10.2. If the extract does not require cleanup, proceed with Sections 10.1.6 and 10.1.7.

**10.1.6** Add 50 mL of methanol or acetonitrile and a clean boiling chip to the flask and repeat the concentration as described above. When the apparent volume of the liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methanol or acetonitrile. A 5-mL syringe is recommended for this operation.

**10.1.7** Add a clean boiling chip to the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methanol or acetonitrile to the top. Place the micro K-D apparatus on a hot water bath (80-85°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methanol or acetonitrile. Adjust the volume to 1.0 mL with methanol or acetonitrile. Add 1.0 mL of reagent water to the extract if methanol or 1.5 mL of reagent water to the extract if acetonitrile (Table 1).

*NOTE: At high concentrations (approximately 1,000 mg/L or greater) of biphenyl in the extract, low recoveries may be obtained due to insolubility in the acetonitrile. Larger volumes of acetonitrile or acetone may be required to dissolve all the biphenyl and to prevent precipitation.*

**10.1.8** Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

**10.2** Cleanup and separation.

**10.2.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

**10.2.2** Prior to HPLC analysis, the composition of the extracts must be as specified under chromatographic conditions in Table 1 and described in Sections 10.1.6 and 10.1.7.

**10.2.3** Proceed with liquid chromatography as described in Section 10.3.

**10.3** Liquid chromatography analysis.

**10.3.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention times and estimated detection limits that can be achieved by this method. An example of the separation achieved by Column 1 of the analytes in a POTW extract is shown in Figure 1. Other columns, chromatographic conditions,[8] or detectors may be used if data quality comparable to Table 2 is achieved.

**10.3.2** Calibrate the system daily as described in Section 8.

**10.3.3** Inject 50 µL of the sample extract. Monitor the column eluent at 254 nm. Record the resulting peak size in area or peak height units.

**10.3.4** The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

**10.3.5** If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.

**10.3.6** If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

## *11. CALCULATIONS*

**11.1** Determine the concentration of analytes in the sample.

**11.1.1** Calculate the amount of analytes injected from the peak response using the calibration curve or calibration factor in Section 8.2.2. The concentration in the sample can be calculated from the equation:

***Equation 3***

$$\textit{Concentration, } \mu g/L = \frac{(A)\ (V_t)}{(V_i)\ (V_s)}$$

*where*

$A$ = *Amount of analytes injected, in ng*

$V_i$ = *Volume of extract injected, in µL*

$V_t$ = *Volume of total extract, in µg/L*

$V_s$ = *Volume of water extracted, in mL*

## *12. METHOD PERFORMANCE*

**12.1** The EDLs and associated chromatographic conditions for the analytes are listed in Table 1. The EDL is defined as the minimum response being equal to 5 times the background noise, assuming a 2-mL final extract volume of a 1-L sample and an HPLC injection volume of 50 µL.

**12.2** Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,[6] in the designated matrices. The results of these studies are presented in Table 2.

# *References*

1. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1980.

2. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

3. "OSHA Safety and Health Standards, General Industry" (29 *CFR* 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

5. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

6. Test procedures for Pesticides in Wastewaters, EPA Contract Report No. 68-03-2897. Unpublished report available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio, March 1979.

8. Beernaert, H. "Determination of Biphenyl and Ortho-Phenylphenol in Citrus Fruits by Gas Chromatography," *Journal of Chromatography*, 77: 331-8, 1973.

9. "Evaluation of Ten Pesticide Methods" U.S. Environmental Protection Agency, Contract No. 68-03-1760, Task No. 11, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Table 1. Chromatographic Conditions and Estimated Detection Limits

| *Parameter* | *Retention Time (min)* | | *Estimated Detection Limit (µg/L)* |
|---|---|---|---|
| | *Column 1* | *Column 2* | |
| o-Phenylphenol | 7.7 | 11.3 | .01 |
| Biphenyl | 18.8 | 16.5 | .04 |

Column 1: Reverse-phase column, 4.6 mm ID by 250 mm long; 10 µ, Perkin-Elmer HC-ODS Sil-X or equivalent; isocratic elution for 5 minutes using 40% acetonitrile in water, then linear gradient elution to 100% acetonitrile over 25 minutes; flow rate of 0.5 mL/min.

Column 2: Reverse-phase column, 4.6 mm ID by 250 mm long; 6-7 µ, Dupont Zorbax ODS or equivalent; isocratic elution for 3 minutes using 50% methanol in water, then linear gradient to 80% methanol over 10 minutes; flow rate 1.0 mL/min.

Table 2. Single-Operator Accuracy and Precision*

| *Parameter* | *Spike Range (µg/L)* | *Number of Replicates* | *Average Percent Recovery*** | *Standard Deviation (%)* |
|---|---|---|---|---|
| o-Phenylphenol | 2.5 | 7 | 102.3 | 36.3 |
| | 6,500 | 7 | 94.1 | 6.3 |
| Biphenyl | 2.4 | 7 | 86.3 | 16.2 |
| | 6,300 | 7 | 100.7 | 9.9 |

*POTW effluent was used in this study.
**No cleanup was employed in validation studies.

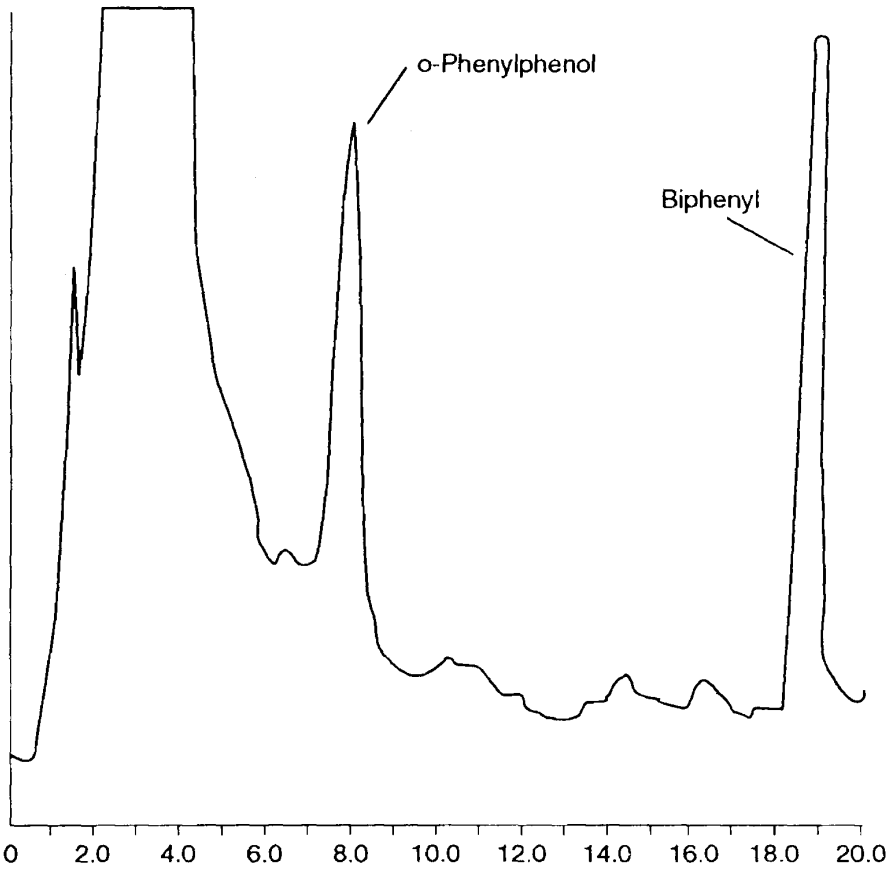


Figure 1. Liquid Chromatogram of Wastewater Extract Fortified With o-Phenylphenol and Biphenyl (Column 1)