NCASI METHOD CP-86.07

CHLORINATED PHENOLICS IN WATER BY *IN SITU* ACETYLATION AND GC/MS DETERMINATION

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Method Status

This method was interlaboratory validated and approved for complying with the monitoring and analytical requirements of the Effluent Limitations Guidelines portion of EPA's "Cluster Rule" in a March 12, 2007, *Federal Register* [*FR 72* (47) 11200-11249] announcement. This final ruling modifies and approves new and alternative test procedures approved for analysis and sampling under the Clean Water Act (CWA) and Safe Drinking Water Act. This method was approved by EPA as an alternative to EPA Method 1653 for the determination of chlorinated phenolics.

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CHLORINATED PHENOLICS IN WATER BY *IN SITU* ACETYLATION AND GC/MS DETERMINATION

1.0 Scope and Application

1.1 Method CP-86.07 is used to determine and confirm the concentrations of selected chlorinated phenols, chlorinated guaiacols, chlorinated catechols, chlorinated benzaldehydes (i.e., vanillins and syringaldehydes), and trichlorosyringol in extracts prepared from water samples by gas chromatography/mass spectrometry (GC/MS). This method is an updated version of NCASI Method CP-86.02 (NCASI 1986, 1997) which has been revised to meet the needs of the pulp and paper industry for the monitoring of chlorinated phenolics for the Cluster Rule (Federal Register 1998). Modifications to the method include: (a) a reduced analyte list (nonchlorinated compounds removed); (b) the addition of acenaphthene–d_{10} as an internal standard for quantitation of 3,4,5-trichlorophenol (*in situ* derivatization internal standard), 3,4,5-trichlorocatechol-$^{13}C_6$ (surrogate), and 5-bromovanillin (surrogate); (c) the use of potassium carbonate to adjust sample pH; (d) the addition of 5-bromovanillin as a surrogate for the chlorovanillins; and (e) the addition of 3,4,5-trichlorocatechol-$^{13}C_6$ as a surrogate for the chlorocatechols. Section 17, Table 1 lists the compounds which can be confirmed or determined by this method.

1.2 This method has been used to analyze untreated and biologically treated pulp mill effluents, influents to treatment plants, and in-process streams. Although this method has been demonstrated to be applicable to many matrix types, each new matrix type (changes in technology may influence the chemical composition of a matrix) should be evaluated for extraction efficiency and method performance. Chlorinated catechol recoveries appear to be influenced by matrix effects (Louch and LaFleur 1998).

1.3 This method has been validated at the multi-laboratory level, and is proposed as an alternative to EPA Method 1653 (Federal Register 1998).

1.4 The concentration range of the calibration curve is from 1.2 to 100 μg/L. The sample size adjusted lower calibration level is dependent on matrix-specific dilutions required to avoid saturation of the detector and to bring the concentration within the calibration range of the instrument. Multi-laboratory method detection limit studies were performed on reagent water and two flow proportioned composites of acid and alkaline filtrates from two facilities equipped with 100% chlorine dioxide substitution bleach plants. The procedure used is from the Federal Register, Volume 49, Appendix B to Part 136, "Definition and Procedure for the Determination of the Method Detection Limit" (Federal Register 1984). The method detection limits determined from the results of these studies are presented in Section 17, Table 2. Because these values were established on a selected group of flow proportioned acid and alkaline sewers from two sources, the reported method detection limits may not be applicable to all matrices. Therefore, the laboratory should establish method detection limits for each sample matrix type.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatography and mass spectrometry, and skilled in the interpretation of chromatograms and mass spectra.

1.6 Any modification of the method beyond those expressly permitted is subject to the application and approval of alternative test procedures under 40 CFR 136.4 and 136.5.

2.0 Summary of Method

2.1 Method CP-86.07 contains the *in situ* derivatization and extraction procedures and the gas chromatographic conditions for the detection of µg/L levels of selected chlorinated phenolics. A 300-mL aliquot of the aqueous sample is spiked with the internal standards and surrogates, neutralized using potassium carbonate, then buffered with potassium carbonate in order to form the phenolate ions at a pH of 9 to 11.5. The phenolate ions are then converted *in situ* (i.e., in the aqueous matrix) to their acetate derivatives by the addition of acetic anhydride. The phenolic acetates thus formed are extracted with hexane.

2.2 A 1-µL portion of the hexane extract (final volume 0.3 mL) is injected into a gas chromatograph using a Grob type splitless injection technique and is chromatographed on a narrow bore fused silica capillary column. The gas chromatography column is temperature programmed to separate the analytes, which are then detected by a mass spectrometer.

2.3 Target analytes are identified by comparing relative retention times and mass spectra with the mass spectra of known standards. The standards used to determine the calibration curve are prepared by spiking the internal standard and the appropriate levels of analytes into blank water and then derivatizing in the same manner as the sample. The analyte is quantitated using the average relative response factor from a five-point calibration curve and internal standard quantitation techniques.

2.4 Quantitative analysis is performed using an internal standard technique with 3,4,5-trichlorophenol as the internal standard for the chlorophenols, chloroguaiacols, trichlorosyringol, chlorocatechols, and chlorovanillins.

2.5 The sensitivity of NCASI Method CP-86.07 depends on both the level of interferences in the matrix and the sensitivity of the GC/MS. Generally, low µg/L detection limits can be achieved in most samples.

2.6 Quality is assured through reproducible calibration and testing of the extraction and analytical (GC/MS) systems. A method blank plus a sample duplicate and matrix spike or matrix spike duplicate are analyzed with each analytical batch (set of no more than 20 samples of a matrix type) to ensure data quality. An instrument internal standard, acenaphthene-d_{10}, is added to each sample to measure the recovery of the internal standard and surrogates, 3,4,5-trichlorophenol, 3,4,5-trichlorocatechol-$^{13}C_6$, and 5-bromovanillin, respectively.

3.0 Definitions

3.1 Chlorinated phenolics are the chlorinated phenols, guaiacols, catechols, vanillins, syringaldehydes, and other compounds amenable to *in situ* acetylation, extraction, and determination by GC/MS using this method.

3.2 The definitions and purposes below are specific to this method, but conform to common usage as much as possible.

3.2.1 µg/L – micrograms per liter

3.2.2 *In situ* acetylation – conversion of the phenolate analytes in the aqueous matrix to the acetate derivatives

3.2.3 May – this action, activity, or procedural step is neither required nor prohibited

3.2.4 Must not – this action, activity, or procedural step is prohibited

3.2.5 Must – this action, activity, or procedural step is required

3.2.6 Should – this action, activity, or procedural step is suggested, but not required

4.0 Interferences

4.1 When a similar procedure (NCASI Method CP-86.01 1986) was applied to groundwater samples collected in the vicinity of a source of creosote (i.e., a wood preservation plant), the high levels of non-chlorinated phenols caused poor recoveries and the method was found unsatisfactory. It is likely that Method CP-86.07 would be subject to the same limitation, although no tests have been run to confirm this.

4.2 The internal standard, 3,4,5-trichlorophenol, has been shown by some researchers to be a persistent anaerobic degradation product of 2,3,4,5-tetrachlorophenol and/or pentachlorophenol. Thus, Method CP-86.07 would have to be modified appropriately when applied to samples suspected of containing these compounds and having undergone anaerobic degradation. Other analysts have used 2,6-dibromophenol as an alternative internal standard in similar *in situ* acetylation GC/ECD procedures.

4.3 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. All these materials must be routinely demonstrated to be free from interferences under the conditions of the analyses by running laboratory blanks as described in Section 9.4. The most frequently identified blank problem is pentachlorophenol contamination. Generally, this has been traced to the potassium carbonate and can be removed by baking the reagent at 400°C for 6 to 8 hours.

4.4 All glassware should be free of interferences. This can be accomplished by washing with hot detergent solution, water rinsing, and air drying. Alternatively, glassware that is washed with hot detergent solution, water rinsed, air dried, and then rinsed with acetone prior to use has been demonstrated to be free of interferences.

4.5 Matrix interferences may be caused by other compounds that are co-extracted from the sample. The extent of these interferences will vary depending upon the matrix and the diversity of the wastewater being sampled. The presence of large concentrations of sulfur in bleach plant filtrate samples can effect chlorocatechol recovery.

4.6 Chlorocatechols are susceptible to degradation by active sites on injection port liners and columns, and are subject to oxidation to the corresponding chloro-o-benzoquinones. A small amount of ascorbic acid may be added to samples to prevent auto-oxidation. For pulp and paper industry samples, ascorbic acid may be added to treated effluent samples only.

5.0 Safety

5.1 The toxicity or carcinogenicity of each analyte or reagent has not been precisely determined; however, each chemical should be treated as a potential health hazard. It is recommended that prudent practices for handling chemicals in the laboratory be employed.

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness of OSHA regulations regarding safe handling of chemicals used in this method. Material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.3 A laboratory fume hood or approved respirator, eye protection, chemical resistant gloves, and protective clothing should be employed. Acetic anhydride is a corrosive, colorless liquid with a pungent odor and may be harmful if swallowed, inhaled, or absorbed through the skin.

6.0 Equipment and Supplies

6.1 Brand names and suppliers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.2 Sampling equipment

6.2.1 Automatic sampling equipment which comes in contact with samples should be constructed of glass, Teflon™, or stainless steel. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing

may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination.

6.2.2 Samples are to be collected in amber glass bottles with Teflon-lined screw caps that are demonstrated to be free of chlorinated phenolics.

6.3 Laboratory glassware and supplies

6.3.1 1-L separatory funnel with Teflon stopcock

6.3.2 500-mL beaker

6.3.3 500-mL and 50-mL graduated cylinders

6.3.4 Volumetric pipets

6.3.5 Volumetric flasks

6.3.6 50-mL centrifuge tubes with Teflon-lined screw caps

6.3.7 2-mL vials with Teflon-lined caps (autosampler vials)

6.3.8 15-mL Kuderna-Danish (KD) concentrator tube

6.3.9 250-mL KD evaporative flask, attach to concentrator tube with springs

6.3.10 Three ball macro Snyder column, KD

6.3.11 Syringes, various sizes

6.3.12 Teflon boiling chips

6.4 Laboratory equipment

6.4.1 pH meter calibrated using two-point procedure

6.4.2 Centrifuge, bench top model, capable of accepting 50-mL centrifuge tubes and achieving 2500 RPM

6.4.3 Evaporation/concentration assembly, Pierce 19797 Uni-Vap Evaporator or equivalent

6.4.4 Water bath – constant temperature capable of heating to 90°C with a temperature control of ±2°C, should be used in a hood

6.4.5 Magnetic stirrer with Teflon-lined stir bar

6.4.6 Analytical balance capable of weighing 0.1 mg

6.4.7 Top loading balance capable of weighing 10 mg

6.5 Analytical equipment and supplies

6.5.1 Gas chromatograph/mass spectrometer (GC/MS): complete with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes, analytical columns, and gases

6.5.2 Gas chromatograph column: 30 m x 0.25 mm bonded-phase fused silica DB-5 capillary column, 0.25 micron film thickness (J&W Scientific DB-5 or equivalent)

6.5.3 Mass spectrometer capable of scanning from 42 to 450 amu every 1 sec or less, utilizing 70 volts (nominal) electron energy in the electron impact ionization mode: A computer system capable of allowing the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be interfaced to the mass spectrometer. The computer must have software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an extracted ion current profile (EICP). Software that allows integrating the abundance in any EICP between specified time or scan number limits must also be available.

6.5.4 Mass spectral libraries: User created libraries containing mass spectra obtained from authentic standards shall be employed to reverse search GC/MS runs for the compounds of interest or for comparison to the spectra determined for the compounds of interest during quantitation review.

6.5.5 Response factors and multi-point calibrations: The data system shall be used to record and maintain lists of response factors (response ratios for isotopic dilution) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial and ongoing performance shall be computed and maintained.

7.0 Reagents and Standards

7.1 All reagents, solvents, and standards used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks initially and with each sample batch (samples started through the extraction process on a given eight-hour shift, to a maximum of 20).

7.2 Reagents and solvents

7.2.1 Hexane, methanol, and acetone – high purity solvents suitable for gas chromatography and pesticide residue analysis

7.2.2 Acetic anhydride 99% – fractionally distilled to eliminate contamination for low-level analyses

7.2.3 Reagent grade water – organic free such as produced by a Barnstead Model D2798 NANO-pure, a water purification system

7.2.4 Potassium carbonate (K_2CO_3) – dissolve 150 g K_2CO_3 (purified by heating at 400°C for 6 to 8 hours in a shallow tray) in 250 mL reagent grade water

7.2.5 Sulfuric acid (H_2SO_4) – 1:4 solution in reagent grade water

7.2.6 Sodium thiosulfate ($Na_2S_2O_3$) solution (1N) – weigh 79 g in a 1-L volumetric flask and dilute to the mark with reagent grade water

7.2.7 Ascorbic acid solution – prepare a solution of ascorbic acid in reagent grade water at a concentration of 0.1 g/mL. This solution must be prepared fresh on each day when derivatizations will be performed; therefore, the volume prepared should be limited to what will be used. This solution is limited to use in effluent samples only.

7.3 Standards

Standards are purchased as solutions or mixtures with certification of their purity, concentration, and authenticity, or are prepared from materials of known purity and composition. If chemical purity of a compound is 98% or greater, the weight may be used without correction to compute the concentration of the standards. If the chemical purity of any standard does not meet the 98% purity requirement the laboratory must correct all calculations, calibrations, etc. for the difference in purity.

7.3.1 Decafluorotriphenylphosphine (DFTPP) can be purchased from Supelco or an equivalent supplier as a 25,000 µg/mL solution in dichloromethane. Prepare a working stock solution at a concentration of 50 µg/mL in dichloromethane. Store in the dark at 4°C in vials with Teflon caps.

7.3.2 3,4,5-Trichlorophenol internal standard stock solution – Weigh (to the nearest 0.1 mg) 40 ±5 mg of 3,4,5-trichlorophenol and dissolve to volume with methanol in a 10-mL volumetric flask (4 mg/ml). Transfer the stock solution into a vial with a Teflon-lined screw cap and store under refrigeration (4°C).

7.3.3 3,4,5-Trichlorophenol internal standard spiking solution – Transfer 0.5 mL of the stock solution (Section 7.3.2) into a 25-mL ground glass stoppered volumetric flask and dilute to volume with methanol (80 µg/mL). Transfer the spiking solution into Teflon-lined screw capped vials and store under refrigeration (4°C).

7.3.4 3,4,5-Trichlorocatechol-$^{13}C_6$ surrogate stock solution – Weigh (to the nearest 0.1 mg) 10 ±1 mg of 3,4,5-trichlorocatechol-$^{13}C_6$ and dissolve to volume with

10.0 mL of methanol (1mg/mL). Store under refrigeration (4°C) in a Teflon-lined screw cap vial.

7.3.5 3,4,5-Trichlorocatechol-$^{13}C_6$ surrogate spiking solution – Transfer 0.25 mL of the stock solution (Section 7.3.4) into a 10-mL ground glass stoppered volumetric flask and dilute to volume with methanol (25 µg/mL). Store under refrigeration (4°C) in a Teflon-lined screw cap vial.

7.3.6 Acenaphthene-d_{10} (99% pure) instrument internal standard – Weigh 20 ±1 mg of acenaphthene-d_{10} and dissolve in hexane in a 25-mL volumetric flask (0.8 mg/mL).

7.3.7 Primary stock solutions – Prepare stock solutions of individual compounds by weighing (to the nearest 0.1 mg) 40 ±5 mg of each compound of a known purity and dissolving in the appropriate solvent in a 10-mL volumetric flask (4 mg/mL). Dissolve the chlorinated phenols, chlorinated catechols, and chlorinated guaiacols in methanol. Dissolve the chlorinated benzaldehydes and the trichlorosyringol in acetone. Chlorinated benzaldehydes are subject to degradation in methanol. Transfer the individual stock solutions to vials with Teflon-lined screw caps and refrigerate at 4°C.

7.3.8 Secondary stock solutions – Prepare two secondary stock mixes by adding 300 µL of each stock solution (Section 7.3.7) to a 50-mL ground glass stoppered volumetric flask in the groups shown in Section 17, Table 3. Bring to volume with methanol or acetone as in the primary stock solutions. The secondary stock concentrations are approximately 25 µg/mL. Alternatively, stocks can be prepared by diluting purchased primary stock solutions or mixes to the appropriate concentrations.

7.3.9 5-Bromovanillin surrogate stock solution – Prepare 5-bromovanillin surrogate primary stock solution in accordance with the directions for the target analyte primary stock in Section 7.3.7 (4 mg/mL). Prepare the 5-bromovanillin solution in acetone.

7.3.10 5-Bromovanillin spiking solution – Prepare the spiking solution by adding 300 µL of the primary stock solution (Section 7.3.9) to a 50-mL ground glass stoppered volumetric flask. Bring to volume with acetone as in the surrogate primary stock solution (25 µg/mL).

7.3.11 Calibration curve standards

7.3.11.1 Stock solutions of all standards should be checked for signs of degradation prior to the preparation of calibration or performance test standards, and must be replaced if a change in concentration is indicated.

7.3.11.2 The calibration curve is prepared by spiking separate 300-mL portions of reagent water with 100 µL of the 3,4,5-trichlorophenol internal standard spiking solution (Section 7.3.3), and 15-, 25-, 100-, 500-, and 1250-µL portions of the calibration spiking solutions (Section 7.3.8) and the surrogate spiking solutions (Section 7.3.10 and Section 7.3.5). The resulting solutions are then acetylated, extracted, and concentrated in a manner analogous to the 300-mL sample aliquots (Section 11.0). The final extract is spiked with 10 µL of acenaphthene-d_{10} solution (Section 7.3.6) prior to transfer to an autosampler vial. This will produce calibration solutions of nominal 1.25, 2.0, 8.0, 40.0, and 100 µg/L of the chlorophenolics and constant concentrations of 25 µg/L of acenapthene-d_{10} and 3,4,5-trichlorophenol per sample. Ascorbic acid is added to samples of final effluents to stabilize the chlorocatechols, but is not added to samples of pulp and paper in-process wastewaters. Therefore, it is necessary to prepare separate sets of calibration curves with and without ascorbic acid when quantitating both types of matrices.

7.3.12 Precision and recovery standards

Initial precision and recovery (IPR) standards are prepared by spiking separate 300-mL portions of reagent water with 100 µL of the 3,4,5-trichlorophenol internal standard spiking solution (Section 7.3.3), 150 µL of the surrogate spiking solutions (Sections 7.3.5 and 7.3.10), and 250 µL of the calibration spiking solutions (Section 7.3.8). The resulting solutions are then acetylated, extracted, and concentrated in a manner analogous to the 300-mL sample aliquots (Section 11.0). The final extract is spiked with 10 µL of acenaphthene–d_{10} solution (Section 7.3.6) prior to transfer to an autosampler vial. This produces IPR solutions with a nominal concentration of 20 µg/L per sample.

8.0 Sample Collection, Preservation, and Storage

8.1 Collection

Grab samples must be collected in glass containers with Teflon-lined screw caps. Composite samples may be collected in refrigerated bottles using automatic sampling equipment, or by grab samples collected at regular intervals throughout the sampling period; i.e., collect one grab sample every 4 hours for a 24-hour composite or as specified by your regulatory authority. Composite samples should be chilled during the sampling period. Collection of two liters of sample will provide sufficient amounts for testing and quality control requirements.

8.2 Preservation

A portion of the sample should be tested for free or residual chlorine. If the presence of residual chlorine is indicated, add 1 mL of sodium thiosulfate solution (1N)

(Section 7.2.6) for every 2.5 part per million (ppm) of free chlorine or until residual chlorine is no longer detected.

All samples must be preserved by adjusting to pH 2 with 1:4 H_2SO_4 (Section 7.2.5) and refrigerating at 4°C. This should be done as soon as possible after sample collection. Samples must be shipped in iced containers as quickly as possible.

8.3 Storage

Samples may be stored in the refrigerator (4°C) for up to 30 days. Acetylated extracts should be maintained at a temperature of approximately 4°C and should be analyzed within 30 days after acetylation and extraction. If the quality control criteria for a sample are not meet and the 30 day holding time is exceeded, a new sample must be collected.

9.0 Quality Control

9.1 Each field sampling program or laboratory that uses this method must operate a formal quality assurance program. Laboratory or field performance is compared to established criteria to determine if the results of the analyses meet the performance characteristics of the method. The laboratory is permitted to modify this method to improve separations or lower the costs of measurement, provided all performance specifications are met. Each time a modification is made to the method, the laboratory is required to repeat the procedures in Sections 9.3 and 10 to demonstrate method performance. If the detection limits for the analytes in this method will be affected by the modification, the laboratory should demonstrate that each MDL is less than or equal to the MDL in this method or one-third the regulatory compliance level, whichever is higher.

9.2 GC/MS performance

9.2.1 Prior to analyzing any samples or standards, GC/MS performance must be verified by a DFTPP tune. This tune check standard (Section 7.3.1) must be analyzed just prior to the calibration standard and meet the specifications listed in Section 17, Table 4.

9.2.2 Calibration verification is performed before each set of samples is analyzed to determine if the GC/MS system is operating within acceptable parameters. The calibration check involves reanalyzing one of the extracts used in the calibration curve. The concentration for each of the analytes must be within ±20% of the initial calibration (Section 10.0). If the calibration fails to meet the expected criteria, the GC/MS system may require maintenance. If routine maintenance does not correct the problem, the instrument will need to be recalibrated.

9.2.2.1 GC resolution: The valley height between 4,6-dichlororguaiacol and 3,4-dichloroguaiacol at m/z 192 shall not exceed 10% of the height of the taller of the two peaks.

9.2.2.2 Each compound injected shall give a single, distinct GC peak.

9.3 Initial demonstration of method proficiency

9.3.1 Initial Precision and Recovery (IPRs)

9.3.1.1 The laboratory shall make an initial demonstration of its ability to generate acceptable results with this method by conducting an assessment of initial precision and recovery by derivatizing, extracting, concentrating, and analyzing four 300-mL aliquots of the precision and recovery standard as described in Section 7.3.12. Separate sets of IPR aliquots must be prepared with and without the addition of ascorbic acid if final effluents and in-process wastewaters from pulp and paper facilities are to be analyzed.

9.3.1.2 Using the results of the four IPR analyses (determined using the procedure in Section 12.0), compute the average percent recovery (%R) and the relative standard deviation (%RSD) of the recoveries for each compound. Compare the %R and %RSD with the corresponding tolerance intervals listed in Section 17, Table 5. These limits were determined based on the results obtained during the multi-laboratory validation of the method.

9.3.2 Method Detection Limit (MDL)

To establish its ability to detect the analytes in this method, the laboratory should determine the MDL in accordance with the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method (Federal Register 1984). MDLs determined in reagent grade water and two flow proportioned combined bleach plant filtrates are listed in Section 17, Table 2. Laboratories should be able to achieve MDLs equal to or less than the listed MDLs or one-third the regulatory compliance level (Section 17, Table 2) for the compounds to be reported prior to the practice of this method.

9.4 Method performance

9.4.1 Frequency

A minimum of one sample per analytical batch, containing no more than 20 samples of similar matrix type, should be allocated for quality control. In addition, representative samples from each new or untested source or sample matrix should be treated as quality control samples. Laboratory replicates and

fortifications (matrix spikes) should be conducted on each QC sample to document method performance as indicated by precision and recovery.

9.4.2 Blanks

A method blank should be performed with each analytical batch of samples to demonstrate that all glassware, reagents, and instrumentation required for sample analyses are free of interferences. New reagents should also be tested prior to processing any samples. The method blanks should be carried through all stages of the sample preparation and measurement, using reagent water as the sample matrix. If any of the compounds of interest or any potentially interfering compounds are found in a blank at greater than 5 µg/L (assuming a response factor of one relative to the sample matrix internal standard for the compounds not listed in Table 1), analysis of samples should be halted until the source of contamination is eliminated.

9.4.3 Replicates

Replicates consist of two or more separate aliquots of the sample run through the entire analytical procedure. The concentration determined for each analyte, the range of concentrations determined by the replicates, and the mean should be tabulated in a method precision log. At a minimum, duplicate measurements should be performed for each analytical batch. Where the analytes are not detected in the sample matrix, duplicate matrix spikes may be performed to measure precision. The relative percent difference of duplicate measurements is calculated in Equation 1.

Equation 1

$$\text{Relative Percent Difference (\%)} = \frac{100 \times (S1 - S2)}{\text{Mean concentration}}$$

Where:
S1 = Sample 1, concentration
S2 = Sample 2, concentration

9.4.4 Matrix spike recovery

Using the mean concentration determined by the replicate analyses or the background level determined by a single measurement, determine the spiking level which will give three to five times the background level. For samples with non-detect background levels, spike the samples at five times the lower calibration level. Spike the sample with the determined amount of the two matrix spiking solutions (Section 7.3.8) and proceed with derivatization, extraction, and analysis of the spiked sample in the normal manner. Calculate the percent recovery for the matrix spike using Equation 2. The analyst should evaluate the data to determine if trends show degradation of standards

or other analytical problems. The average matrix spike recoveries and percent relative standard deviations of recovery presented in Section 17, Table 6 were developed from the multi-laboratory data collected during a Tier 2 ATP study in a bleached plant filtrate sample.

Equation 2

$$\text{Percent Recovery} = \frac{\text{Concentration Measured - Background Concentration}}{\text{Concentration Spiked}} \times 100$$

Each laboratory should establish quality control charts and recovery criteria for specific matrices. Calculate and record the percent recovery as in Equation 2. Compare the matrix spike recovery to the tolerance intervals listed in Section 17, Table 6. If the compounds meet the criteria, method performance is verified. After the analyses of five samples for which the recovery criteria have been met, develop a statement of laboratory accuracy for each compound in each matrix type by computing the average percent recovery (P) and the standard deviation of the percent recovery (sp). Express the accuracy assessment as a percent recovery interval from P–2sp to P+2sp for each matrix. Update the accuracy assessment for each compound on a regular basis.

9.4.5 Internal standard recovery

Determine the recovery of the 3,4,5-trichlorophenol *in situ* internal standard. The concentration of 3,4,5-trichlorophenol is calculated using acenaphthene-d_{10} as the internal standard. The 3,4,5-trichlorophenol internal standard recovery should meet the criteria specified in Section 17, Table 5. If the recovery of 3,4,5-trichlorophenol falls outside the tolerance intervals, method performance is unacceptable for that compound in that sample. Therefore, the sample is complex and may be reanalyzed using the guidance provided in Section 11.2. The analyst should evaluate the data to determine if there are matrix effects, degradation of standards, or instrument problems which should be corrected prior to reanalysis or re-extraction.

9.4.6 Surrogate recovery

In this method 5-bromovanillin is utilized as a surrogate for the aldehydes and 3,4,5-trichlorocatechol-$^{13}C_6$ is utilized as a surrogate for the chlorocatechols. Spike all samples with 150 µL 3,4,5 trichlorocatechol-$^{13}C_6$ spiking solution (Section 7.3.5) and 150 µL 5-bromovanillin spiking solution (Section 7.3.10) to monitor surrogate recovery. Compute the recovery of the surrogate using Equation 3. The concentrations of 5-bromovanillin and 3,4,5-trichlorocatechol-$^{13}C_6$ are determined using acenaphthene-d_{10}.

Equation 3

$$\textit{Surrogate Percent Recovery} = \frac{\textit{Concentration Measured x 100}}{\textit{Concentration Spiked}}$$

Performance criteria for acceptable surrogate recovery, as determined during a multi-laboratory validation of this method, are presented in Section 17, Table 7. If the recovery of the surrogates fall outside the warning limits, method performance is unacceptable for that compound in that sample. Therefore, the sample is complex and should be reanalyzed using the guidance provided in Section 11.2.

10.0 Calibration and Standardization

10.1 GC/MS operating conditions

Establish the operating conditions outlined below for the GC/MS system. Once the GC/MS system is optimized for sensitivity and analyte peak separation, the same operating conditions must be used to analyze all samples, blanks, calibration standards, and quality assurance samples. Each compound injected shall give a single, distinct GC peak.

GC/MS Operating Conditions

Injector Temperature	270°C
Interface Temperature	270 to 280°C
Splitless Valve Time	0.7 to 1.0 min
Carrier Gas	Helium @ 36 cm/sec @ 200°C
Column	DB-5 or RTX-5MS, 30 m x 0.25 mm id x 0.25 micron
Initial	50°C for 1 min
Ramp 1	8°C/min to 270°C, hold 1 min
Post run	325°C for 5 min (this post run is recommended when analyzing high level samples, adjust the hold time as needed for further bakeout)
Scan Start Time	4 min
Scan Range	42 to 450 AMU
Cycle Time	0.8 to 1.2 seconds

10.2 Obtain a mass spectrum of the acetyl derivative for each of the compounds to be analyzed by derivatizing and analyzing an authentic standard either singly or as part of a mixture in which there is no interference between closely eluting components. This library of mass spectra can be utilized to assist in the identification and confirmation of each compound.

10.3 Inject 1 µL of each of the calibration standards (Section 7.3.11) onto the GC/MS. Demonstrate that the target analytes are detectable at the minimum levels using the lowest level calibration curve extract. Calculate the relative response factors using the integrated areas of extracted ion current profiles (EICP) for the characteristic ions shown in Section 17, Table 8. Calculate the relative response factors using Equation 4.

Equation 4

$$RRF = [(A_S/A_{IS}) \times (C_{IS}/C_S)]$$

where:

A_S = *area of the target compound's characteristic mass in the calibration standard*
A_{IS} = *area of the characteristic mass for the internal standard*
C_S = *concentration of the target compound in the calibration standard*
C_{IS} = *concentration of internal standard in the calibration standard*

An example calibration curve for 3,4,6-trichlorocatechol is illustrated in Section 17, Figure 1.

If the average of the relative response factors (RRF) calculated across the calibration range are constant, i.e., exhibit a coefficient of variation <20%, the calibration is acceptable and the average RRF can be used in all subsequent quantifications; otherwise, the calibration curve extracts must be reanalyzed and reevaluated. If reanalysis also fails to produce a constant RRF, new calibration curve extracts must be prepared and analyzed.

10.4 The instrument internal standard, acenaphthene-d_{10}, is used to determine the recoveries of 3,4,5-trichlorocatechol-$^{13}C_6$, 3,4,5-trichlorophenol, and 5-bromovanillin. The recoveries are used to assess quality control as described in Sections 9.4.5 and 9.4.6.

10.5 The calibration curve is verified prior to the analysis of each set of samples (Section 9.2.2). Using a point from the calibration curve, Equation 4 is used to calculate the relative response factor of a calibration check standard daily, prior to sample analysis. Recalibration is required if the relative response factor for the target compounds in the calibration verification point analyzed differs by ±20% of the relative response factor determined for that calibration point in the current calibration curve.

10.6 A blank should be processed with the curve as specified in Section 9.4.2 to confirm that the glassware, reagents, etc. are free from contamination.

11.0 Procedure

11.1 *In situ* acetylation and extraction

11.1.1 Remove the standards and samples from the refrigerator and allow them to come to room temperature. Assemble the necessary glassware.

11.1.2 Calibrate the pH meter using a two-point procedure and pH 7 and 10 buffers.

11.1.3 Shake the sample vigorously to insure it is homogeneous, measure 300 mL using a graduated cylinder, and transfer it to a 500-mL beaker equipped with a magnetic stirrer.

11.1.4 For effluent samples, ascorbic acid is added to stabilize the chlorocatechols. Spike 2 to 3 mL of ascorbic acid solution (Section 7.2.7) into each final effluent sample and the associated calibration standards, IPR, and quality control samples. For pulp and paper industry samples from in-process streams such as acid and alkaline filtrates or untreated effluents, ascorbic acid is not added, thereby preventing the conversion of chloro-o-quinones to chlorocatechols.

11.1.5 Spike all samples, blanks, and quality control samples with 100 µL of 3,4,5-trichlorophenol internal standard solution (Section 7.3.3), 150 µL of 3,4,5-trichlorocatechol-$^{13}C_6$ spiking stock (Section 7.3.5), and 150 µL of 5-bromovanillin spiking stock (Section 7.3.10). Add three to five times the background level or five times the lower calibration level of matrix spiking solution (Section 7.3.8) to the matrix spike quality control samples (Section 9.4.4).

11.1.6 Adjust the pH of the sample to between 7.0 and 7.5 using potassium carbonate solution (Section 7.2.4) while stirring the sample. For calibration standards, IPRs, and blanks, pH adjustment is not required.

11.1.7 Derivatization must proceed rapidly, particularly after the addition of the potassium carbonate solution. Therefore, it is necessary to work with one sample at a time until the derivatization step is complete (Section 11.1.8). Transfer the sample to a 1-L separatory funnel and add 7.8 mL of potassium carbonate solution (Section 7.2.4). Stopper and shake the sample once, quickly. Immediately add 6.0 mL of acetic anhydride (Section 7.2.2) and shake the sample for 30 seconds with frequent venting. Some samples may foam during this step, so it is necessary to use the 1-L separatory funnel to prevent overflow of the sample.

11.1.8 Add 40 mL of hexane and shake vigorously for one to two minutes with frequent venting. Allow the phases to separate (approximately five minutes) and drain the aqueous portion into the beaker. If there is an emulsion, drain the organic layer into a 50-mL centrifuge tube, cap, and centrifuge for two to three minutes or until the emulsion is broken. Transfer as much of the hexane as possible into a 250-mL KD assembly.

11.1.9 Transfer the aqueous portion and residual emulsion back to the separatory funnel and extract two more times with 40 mL of hexane, combining the extracts in the KD.

11.1.10 Add one or two Teflon boiling chips to the KD assembly and pre wet the KD with approximately 1 mL of hexane. Concentration the sample to between 5 and 10 mL over a period of 15 or 20 minutes. Do not allow the sample to go to dryness. Remove the KD assembly from the bath and cool for at least 10 minutes. Remove the Snyder column and rinse down the flask with 1 to 2 mL of hexane. Remove the flask.

11.1.11 Further concentrate the extract in the KD thimble to a volume of approximately 1 mL using a nitrogen blow down apparatus. Add 10 µL of acenaphthene–d_{10} (7.3.6). Transfer the contents to an autosampler vial for analysis and adjust the final volume to 0.3 mL using nitrogen blow down. Do not allow the extract to go to dryness.

11.2 Complex samples

Some samples may contain high levels of interfering compounds and/or other phenolic materials. It is possible that some samples will not concentrate to 0.3 mL, may overload the GC column and/or mass spectrometer, or may exceed the capacity of the derivatizing agent.

11.2.1 For samples that are expected to be difficult to derivatize, concentrate, or are expected to overload the GC column or mass spectrometer, sample dilution may be required. However, to ensure adequate sensitivity, a 300-mL aliquot must always be prepared and analyzed.

11.2.3 Sample extracts containing high levels of sulfur may be treated with activated copper to remove sulfur prior to analysis.

12.0 Data Analysis and Calculations

12.1 Sample analysis

12.1.1 Analyze the sample extract using the conditions described in Section 10.1. Tabulate the extracted ion current profile areas for each of the ions listed in Section 17, Table 8.

12.1.2 Identify the analytes by comparison of the relative retention times established in the calibration to the retention times in the sample. Relative retention time ranges observed during the multi-laboratory validation of this method are listed in Section 17, Table 8. Confirm that the target compound's characteristic m/z signals identified in the reference spectra of the target compound are present and maximized within the same two consecutive scans.

12.1.3 The sample compound relative retention time (RRT) must fall within ±0.06 RRT units of the RRT of the standard compound. The RRT should be assigned by using the EICPs for ions unique to the compound of interest.

12.1.4 The m/z's present in the mass spectrum from components in the sample that are not present in the reference spectrum should be accounted for by contamination or background ions. If the experimental mass spectrum is contaminated or identification is ambiguous, the analysis and/or derivatization and sample extraction should be repeated.

12.1.5 Calculate the sample concentration, using the relative response factors established in Section 10.3 as indicated according to Equation 5.

Equation 5

$$Concentration\ (\mu g/L) = (A_s \times C_{IS})/(A_{IS} \times RRF)$$

where:
A_s = *area of the characteristic ion of the target analyte*
C_{IS} = *concentration of the internal standard µg/L*
A_{IS} = *area of the characteristic ion of the internal standard peak*
RRF = *relative response factor of the target analyte (Section 10.3)*

12.2 Data review requirements

12.2.1 Review the data for accuracy of the identification, GC problems, interferences, and bias. Correct any problems prior to reporting analytical results.

12.2.2 Manually review all the chromatograms to confirm internal standard and analyte identification and area integrations. As part of this review, assess whether or not the concentration is within the calibration range of the instrument. Determine if the level of interferences and baseline noise can be corrected with dilution of the extract. Low recoveries of surrogates and high baseline noise are often indicators of matrix effects, and if they are present the sample may need to be diluted and re-extracted.

12.2.3 Resolve any inconsistencies between replicate analyses (i.e., if a compound is detected in one replicate and not the other), and attempt to determine the cause.

12.2.4 Generate a report that includes the retention time of the compound, the area of the characteristic ion (GC/MS), and the calculated concentration of the target compound detected in µg/L.

12.3 Data reporting requirements

12.3.1 Report results in µg/L to one decimal place.

12.3.2 Report all corresponding blanks, replicates, surrogate recoveries, and matrix spike recoveries for each analytical batch of samples.

13.0 Method Performance

13.1 Verify that the quality control criteria outlined in Section 9.0 have been attained.

13.2 A chromatogram of the mid-point in the calibration curve (Section 7.3.11) is shown in Section 17, Figure 2.

14.0 Pollution Prevention

The laboratory should check state and local requirements to determine if pollution prevention equipment, such as solvent recovery devices, are required or recommended in its area. Use of these devises to reclaim solvents can be part of a pollution prevention program to reduce air emissions.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing releases into the environment. Compliance with all sewage discharge permits and regulations is also required.

15.2 Instructions for sample and waste handling and disposal

15.2.1 All flammable waste solvents must be handled under federal, state, and local guidelines for proper disposal.

15.2.2 Acetic anhydride may be slowly reacted with baking soda to a pH greater than 5 in a large beaker before being poured down the drain with copious amounts of water.

15.2.3 Samples preserved with H_2SO_4 to pH <2 are hazardous and must be neutralized before being disposed, or must be handled as hazardous waste. The aqueous portion of the extracted sample (~pH 7) aliquot may be poured down the drain with copious amounts of water.

15.2.4 For further information on waste management, EPA recommends consulting *The Waste Management Manual for Laboratory Personnel*, and *Less is Better: Laboratory Chemical Management for Waste Reduction*. Both are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington DC, 20036.

16.0 References

16.1 Federal Register. 1998. National Emissions Standards for Hazardous Air Pollutants for Source Category: Pulp and Paper Production; Effluent Limitations Guidelines,

Pretreatment Standards, and New Source Performance Standards; Pulp, Paper, and Paperboard Category; Final Rule. 40 CFR Parts 63, 261, and 430, Wednesday, April 15, 1998.

16.2 Federal Register. 1984. Rules and Regulation, Appendix B to Part 136, Definition and Procedure for the Determination of the Method Detection Limit- Revision 1.11. Vol. 49, No. 209, October 26, 1984.

16.3 Louch, J.R., and LaFleur, L.E. 1998. Experiences with Isotopic Dilution Measurements of Chlorinated Phenolics Using EPA 1653. In: *Chlorine and Chlorine Compounds in the Paper Industry*. Victor Turoski (Ed.), Ann Arbor Press, pp 69-72.

16.4 National Council for Air and Stream Improvement, Inc. (NCASI). 1986. *NCASI methods for the analysis of chlorinated phenolics in pulp industry wastewaters, Method CP-86.01*. Technical Bulletin No. 498. Research Triangle Park, NC: National Council for Air and Stream Improvement, Inc.

16.5 National Council for Air and Stream Improvement, Inc. (NCASI). 1997. NCASI method CP-86.02 chlorinated phenolics in water by in situ acetylation and GC/MS determination. *NCASI Methods Manual*. Research Triangle Park, NC: National Council for Air and Stream Improvement, Inc.

17.0 Tables, Figures, and Validation Data

Table 1. Chlorophenolic Compounds Determined by NCASI CP-86.07

Chlorinated Phenols	CAS Number	Chlorinated Catechols	CAS Number
4-chlorophenol	106-48-9	4-chlorocatechol	2138-22-9
2,4-dichlorophenol	120-83-2	3,4-dichlorocatechol	3978-67-4
2,6-dichlorophenol	87-65-0	3,6-dichlorocatechol	3938-16-7
2,4,5-trichlorophenol	95-95-4	4,5-dichlorocatechol	3428-24-8
2,4,6-trichlorophenol	88-06-2	3,4,5-trichlorocatechol	56961-20-7
2,3,4,6-tetrachlorophenol	58-90-2	3,4,6-trichlorocatechol	32139-72-3
pentachlorophenol	87-86-5	tetrachlorocatechol	1198-55-6
Chlorinated Guaiacols	**CAS Number**	**Chlorinated Aldehydes**	**CAS Number**
4-chloroguaiacol	16766-30-6	5-chlorovanillin	19463-48-0
3,4-dichloroguaiacol	77102-94-4	6-chlorovanillin	18268-76-3
4,5-dichloroguaiacol	2460-49-3	5,6-dichlorovanillin	18268-69-4
4,6-dichloroguaiacol	16766-31-7	2-chlorosyringaldehyde	76341-69-0
3,4,5-trichloroguaiacol	57057-83-7	2,6-dichlorosyringaldehyde	76330-06-8
3,4,6-trichloroguaiacol	60712-44-9		
4,5,6-trichloroguaiacol	2668-24-8		
tetrachloroguaiacol	2539-17-5		
Chlorinated Syringol	**CAS Number**	**Internal Standard (IS)**	**CAS Number**
trichlorosyringol	2539-26-6	3,4,5-trichlorophenol	609-19-8
Instrument IS	**CAS Number**	**Surrogates**	**CAS Number**
acenaphthene-d_{10}	15067-26-2	5-bromovanillin	2973-76-4
		3,4,5-trichlorocatechol-$^{13}C_6$	

Table 2. Method Detection Limits[a]

Compound	Minimum Level (µg/L)	MDL[b] Reagent Water (µg/L)	MDL[b] Range (µg/L)	MDL[c] Bleach Plant Filtrate A (µg/L)	MDL[d] Bleach Plant Filtrate B (µg/L)
4-chlorophenol	1.25	0.32	0.13-0.62	0.47	0.11
2,6-dichlorophenol	2.5	0.29	0.12-0.44	0.09	0.36
2,4-dichlorophenol	2.5	0.22	0.08-0.34	0.32	0.24
2,4,6-trichlorophenol	2.5	0.21	0.12-0.30	0.50	0.64
2,4,5-trichlorophenol	2.5	0.16	0.12-0.21	0.33	0.36
2,3,4,6-tetrachlorophenol	2.5	0.21	0.14-0.33	0.24	0.20
pentachlorophenol	5.0	0.29	0.26-0.31	0.33	0.57
4-chloroguaiacol	1.25	0.16	0.08-0.21	NA	0.54
4,6-dichloroguaiacol	2.5	0.18	0.10-0.24	0.26	0.18
3,4-dichloroguaiacol	2.5	0.16	0.09-0.20	0.15	0.48
4,5-dichloroguaiacol	2.5	0.17	0.09-0.27	NA	0.33
3,4,6-trichloroguaiacol	2.5	0.21	0.09-0.29	0.30	0.19
3,4,5-trichloroguaiacol	2.5	0.19	0.12-0.35	0.38	0.37
4,5,6-trichloroguaiacol	2.5	0.28	0.10-0.44	0.21	0.27
tetrachloroguaiacol	5.0	0.24	0.18-0.35	0.39	0.44
4-chlorocatechol	1.25	0.27	0.21-0.34	0.53	0.39
3,4-dichlorocatechol	2.5	0.24	0.11-0.51	NA	0.76
3,6-dichlorocatechol	2.5	0.24	0.14-0.42	0.38	0.44
4,5-dichlorocatechol	2.5	0.28	0.19-0.55	0.26	0.62
3,4,5-trichlorocatechol	5.0	0.36	0.13-0.81	0.50	0.82
3,4,6-trichlorocatechol	5.0	0.29	0.08-0.54	0.40	0.51
tetrachlorocatechol	5.0	0.33	0.13-0.75	0.34	0.86
5-chlorovanillin	2.5	0.19	0.17-0.24	NA	0.67
6-chlorovanillin	2.5	0.24	0.17-0.35	NA	NA
5,6-dichlorovanillin	5.0	0.26	0.20-0.31	0.89	0.38
2-chlorosyringaldehyde	2.5	0.23	0.19-0.29	0.51	NA
2,6-dichlorosyringaldehyde	5.0	0.39	0.20-0.64	0.30	0.49
trichlorosyringol	2.5	0.27	0.16-0.37	0.15	0.26

[a] MDL results were calculated using the procedure outlined in the Federal Register, Volume 49, Appendix B, Part 136

[b] Average method detection limits from the single laboratory validation study and the four laboratories participating in the multi-laboratory study and the observed range

[c] Method detection limit study was performed using eight replicates of a flow proportioned combined bleach plant filtrate from Mill A using 100% chlorine dioxide bleaching and oxygen delignification spiked at approximately 2 µg/L

[d] Method detection limit study was performed using eight replicates of a flow proportioned combined bleach plant filtrate from Mill B using 100% chlorine dioxide bleaching and oxygen delignification spiked at approximately 2 µg/L

NA - not available due to native concentrations in the sample more than two times higher than the concentrations spiked

Table 3. Secondary Stock Solution Mixes for Target Analytes

Mix #1 Chlorinated Phenols (prepared in methanol)	Chlorinated Catechols (prepared in methanol)
4-chlorophenol	4-chlorocatechol
2,6-dichlorophenol	3,6-dichlorocatechol
2,4-dichlorophenol	3,4-dichlorocatechol
2,4,6-trichlorophenol	4,5-dichlorocatechol
2,4,5-trichlorophenol	3,4,5-trichlorocatechol
2,3,4,6-tetrachlorophenol	3,4,6-trichlorocatechol
pentachlorophenol	tetrachlorocatechol
Chlorinated Guaiacols (prepared in methanol)	**Mix #2** Chlorinated Benzaldehydes (prepared in acetone)
4-chloroguaiacol	6-chlorovanillin
4,5-dichloroguaiacol	5-chlorovanillin
4,6-dichloroguaiacol	5,6-dichlorovanillin
3,4-dichloroguaiacol	2-chlorosyringaldehyde
3,4,6-trichloroguaiacol	2,6-dichlorosyringaldehyde
3,4,5-trichloroguaiacol	trichlorosyringol
4,5,6-trichloroguaiacol	
tetrachloroguaiacol	

Table 4. DFTPP Key Masses and Abundance Criteria[a,b]

M/Z	Ion Abundance Criteria
51	8 to 82% of m/z 198
68	less than 2% of m/z 69
69	11 to 91% of m/z 198
70	less than 2% of m/z 69
127	32 to 59% of m/z 198
197	less than 1% of m/z 198
198	base peak, 100% relative abundance
199	4 to 9% of m/z 198
275	11 to 30% of m/z 198
441	44 to 110% of m/z 443
442	30 to 86% of m/z 198
443	14 to 24% of m/z 442

[a] J.W. Eichelberger, L.E. Harris, and W.L. Budde. 1975. Reference compound to calibrate ion abundance measurement in gas chromatography-mass spectrometry. *Analytical Chemistry* 47:995

[b] Suggested parameters for DFTPP analysis – 50 ng DFTPP injected using a Grob type splitless injection and the following gas chromatographic conditions: injection port temperature 210 to 270°C, oven programmed from 150°C after a one minute hold at 10°C/minute to 205°C, followed by a 6 minute hold; mass spectrometer conditions are set to scan from 42 to 500 amu at 216.7 amu/sec

Table 5. Initial Precision and Recovery

Compound[a]	Average Recovery[b] (%)	% RSD[c] Upper Limit	% Recovery Tolerance Interval[d]
4-chlorophenol	105	7.9	85 – 124
2,6-dichlorophenol	102	8.9	86 – 119
2,4-dichlorophenol	104	6.7	80 – 128
2,4,6-trichlorophenol	102	6.8	94 – 110
2,4,5-trichlorophenol	105	6.9	84 – 125
2,3,4,6-tetrachlorophenol	104	4.1	73 – 135
pentachlorophenol	102	12.4	47 – 156
4-chloroguaiacol	102	10.6	78 – 126
4,6-dichloroguaiacol	105	6.5	65 – 144
3,4-dichloroguaiacol	105	9.0	74 – 135
4,5-dichloroguaiacol	104	5.6	88 – 120
3,4,6-trichloroguaiacol	104	6.0	87 – 121
3,4,5-trichloroguaiacol	103	4.7	60 – 146
4,5,6-trichloroguaiacol	103	10.8	62 – 145
tetrachloroguaiacol	101	6.5	58 – 145
4-chlorocatechol	105	16.2	90 – 119
3,6-dichlorocatechol	107	4.4	66 – 149
3,4-dichlorocatechol	108	11.1	80 – 134
4,5-dichlorocatechol	104	4.1	55 – 153
3,4,5-trichlorocatechol	102	8.1	43 – 162
3,4,6-trichlorocatechol	105	12.8	63 – 148
tetrachlorocatechol	103	5.5	44 – 164
5-chlorovanillin	106	6.6	80 – 131
6-chlorovanillin	107	4.0	69 – 145
5,6-dichlorovanillin	107	6.5	71 – 142
2-chlorosyringaldehyde	107	17.1	72 – 141
2,6-dichlorosyringaldehyde	104	9.6	46 – 163
trichlorosyringol	102	15.8	54 – 151
IS and Surrogates[e]			
3,4,5-trichlorophenol	103	8.1	46 – 160
3,4,5-trichlorocatechol-$^{13}C_6$	110	18.2	52 – 169
5-bromovanillin	110	21.3	32 – 188

[a] 3,4,5-Trichlorophenol used as the quantitation internal standard

[b] Pooled average of the IPR average results of four replicates (~20 µg/L spike) in reagent grade water analyzed at four different laboratories

[c] Upper tolerance interval for pooled percent relative standard deviations, p = 0.95, r = 0.95, k = 6.37, n = 4

[d] Data from Tier 2 multi-laboratory validation of NCASI Method CP-86.07 based on tolerance intervals, p = 0.95, r = 0.95, k = 6.37, n = 4

[e] Acenaphthene-d_{10} used as the quantitation internal standard

Table 6. Matrix Spike Recovery Multi-Laboratory Validation Data[a]

Compound[b]	Average Recovery (%)	RSD %	% Recovery Tolerance Interval[c]
4-chlorophenol	107	16.6	70 – 143
2,6-dichlorophenol	108	15.3	74 – 142
2,4-dichlorophenol	111	16.1	75 – 148
2,4,6-trichlorophenol	107	13.1	78 – 136
2,4,5-trichlorophenol	102	14.5	72 – 132
2,3,4,6-tetrachlorophenol	97	10.1	77 – 118
pentachlorophenol	85	20.2	50 – 120
4-chloroguaiacol	103	6.0	89 – 116
4,6-dichloroguaiacol	113	10.1	88 – 138
3,4-dichloroguaiacol	130	24	54 – 206
4,5-dichloroguaiacol	116	8.6	96 – 137
3,4,6-trichloroguaiacol	106	6.3	92 – 119
3,4,5-trichloroguaiacol	97	10.6	76 – 118
4,5,6-trichloroguaiacol	101	7.4	85 – 116
tetrachloroguaiacol	93	12.1	70 – 116
4-chlorocatechol	86	11.7	65 – 107
3,6-dichlorocatechol	89	27.7	38 – 140
3,4-dichlorocatechol	81	30.2	31 – 131
4,5-dichlorocatechol	75	31.5	27 – 124
3,4,5-trichlorocatechol	62	47.8	1 – 123
3,4,6-trichlorocatechol	74	37.8	17 – 131
tetrachlorocatechol	57	56	0 – 122
5-chlorovanillin	97	9.7	79 – 118
6-chlorovanillin	87	21.9	49 – 128
5,6-dichlorovanillin	89	13.8	64 – 115
2-chlorosyringaldehyde	93	12.1	70 – 117
2,6-dichlorosyringaldehyde	86	28.0	37 – 136
trichlorosyringol	101	4.7	90 – 110
IS[d]			
3,4,5-trichlorophenol	96	15.5	74 – 118

[a] Data are from a multi-laboratory validation of NCASI Method CP-86.07 at the Tier 2 level with four participating laboratories

[b] Determined for the acetates using 3,4,5-trichlorophenol as the quantitation internal standard

[c] Tolerance intervals were calculated using matrix spike and matrix spike duplicate data collected from the four laboratories participating in the Tier 2 study in a bleach plant filtrate matrix using a p = 0.95, r = 0.95, k = 3.73 for n =8, k = 4.01 for n =7, the k value was adjusted by 10% to account for single test variability.

[d] Determined using acenaphthene-d_{10} as the quantitation internal standard with data for bleach plant filtrate matrices only, n = 15, p = 0.95, r = 0.95, k = 2.95.

Table 7. Surrogate Recovery[a]

Compound[b]	Average Recovery[c] (%) [RSD%]	Warning Limits[c]
3,4,5-trichlorocatechol-$^{13}C_6$	96 [34]	32 - 161
5-bromovanillin	104 [23]	55 - 152

[a] Data from a multi-laboratory validation of NCASI Method CP-86.07

[b] Spike concentration of approximately 12.5 μg/L

[c] Determined using acenaphthene-d_{10} as the quantitation internal standard using the average ± 2*standard deviation

Table 8. Quantitation Ions and Relative Retention Times

Compound	Quantitation Ions	Suggested Qualification Ions	RRT Range[a]	Pooled RRT[b] RSD %	Average RRT[c]
4-chlorophenol	128	130, 170	0.637 – 0.696	2.9	0.674
2,6-dichlorophenol	162	164, 204	0.745 – 0.789	1.9	0.772
2,4-dichlorophenol	162	164, 204	0.752 – 0.809	1.7	0.793
4-chloroguaiacol	158	160, 143, 145	0.859 – 0.887	0.8	0.872
2,4,6-trichlorophenol	196	198, 200, 238	0.874 – 0.898	0.8	0.889
2,4,5-trichlorophenol	196	198, 200, 238	0.938 – 0.962	0.5	0.946
4-chlorocatechol	144	146, 186	0.969 – 1.003	0.6	0.972
4,6-dichloroguaiacol	192	194, 177, 179	0.973 – 0.988	0.2	0.986
3,4-dichloroguaiacol	192	194, 177, 179	0.987 – 0.994	0.1	0.993
3,4,5-trichlorophenol (IS)	196	198, 200, 238			
4,5-dichloroguaiacol	192	194, 177, 179	1.031 – 1.043	0.3	1.034
3,6-dichlorocatechol	178	180, 220, 222	0.985 – 1.085	1.1	1.046
2,3,4,6-tetrachlorophenol	232	234, 230, 274	1.057 – 1.069	0.3	1.062
5-chlorovanillin	186	188, 185, 187	1.066 – 1.087	0.6	1.072
3,4,6-trichloroguaiacol	226	228, 230, 211	1.071 – 1.088	1.0	1.077
6-chlorovanillin	186	188, 185, 187	1.068 – 1.097	0.7	1.081
3,4-dichlorocatechol	178	180, 200, 220	1.086 – 1.141	1.0	1.097
4,5-dichlorocatechol	178	180, 200, 220	1.105 – 1.129	0.7	1.114
3,4,5-trichloroguaiacol	226	228, 230, 211	1.122 – 1.150	0.8	1.132
5-bromovanillin (S)	230	232	1.134 – 1.168	0.9	1.145
4,5,6-trichloroguaiacol	226	228, 230, 211	1.132 – 1.186	1.1	1.162
3,4,6-trichlorocatechol	212	214, 216, 254	1.154 – 1.186	1.0	1.165
5,6-dichlorovanillin	220	222, 219, 221	1.183 – 1.226	1.2	1.198
2-chlorosyringaldehyde	216	218, 215, 217	1.191 – 1.323	1.8	1.210
3,4,5-trichlorocatechol-$^{13}C_6$ (S)	220	218,222	1.201 – 1.249	1.2	1.217
3,4,5-trichlorocatechol	212	214, 216, 254	1.201 – 1.244	1.2	1.244
pentachlorophenol	266	268, 264	1.217 – 1.267	1.2	1.238
tetrachloroguaiacol	262	264, 260, 247	1.240 – 1.245	1.3	1.242
trichlorosyringol	256	258, 260, 241	1.231 – 1.282	1.4	1.250
tetrachlorocatechol	248	250, 246, 290	1.288 – 1.382	1.9	1.313
2,6-dichlorosyringaldehyde	250	252, 249, 251	1.299 – 1.370	1.7	1.324

[a] Retention times of acetate derivatives relative to the internal standard (IS), 3,4,5-trichlorophenol acetate; under the chromatographic conditions in Section 10, the average retention time for 3,4,5-trichlorophenol acetate was 16.97 minutes, with a warning limits of 16.94 to 17.00 during a single laboratory validation of the method

[b] The pooled relative standard deviation for relative retention times based on 3,4,5-trichlorophenol (IS) acetate calculated from multi-laboratory study data collected at four participating laboratories during a Tier 2 ATP study

[c] The upper and lower warning limits calculated using multi-laboratory study data collected at four participating laboratories during a Tier 2 alternative test procedure study, lower limit =average-2*standard deviation (STD), upper limit = average + 2*STD

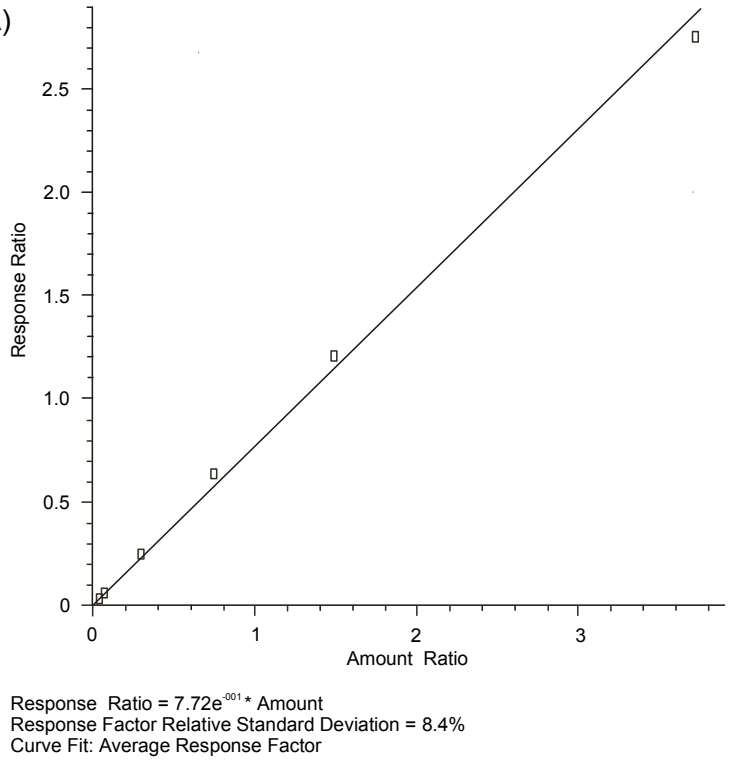

Figure 1. Relative Response Factor Curve for 3,4,6-Trichlorocatechol; 3,4,5-Trichlorophenol as the Internal Standard

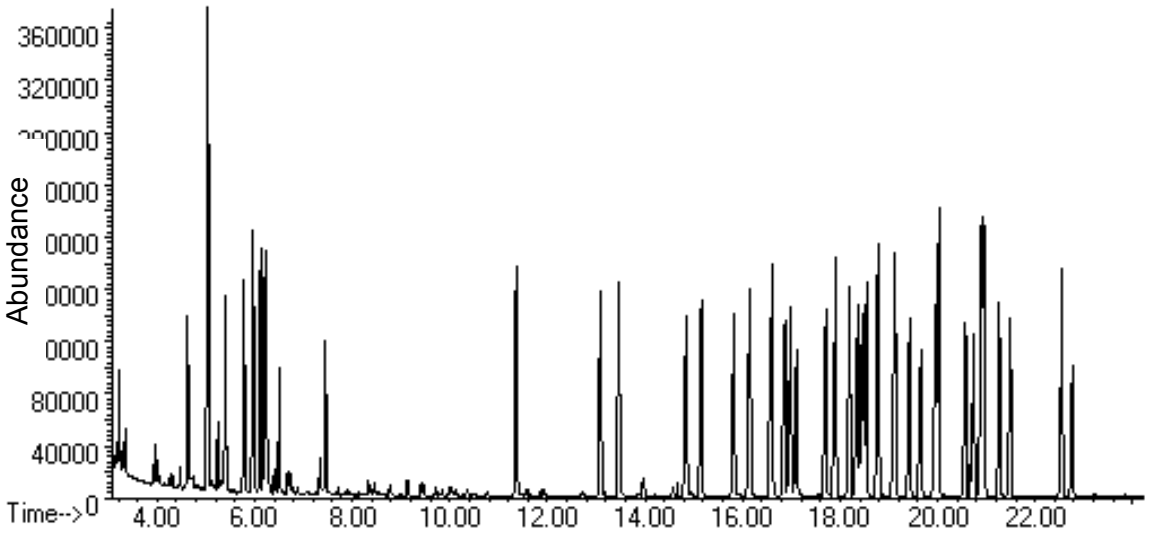

Figure 2. Chromatogram of a Mid-Point Calibration Standard