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Agency

Office of Water
Engineering and Analysis Division (4303)
Washington, DC 20460

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EPA

Analytical Methods for the Determination of Pollutants in Pulp and Paper Industry Wastewater

Acknowledgments

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Disclaimer

This methods compendium has been reviewed by the Engineering and Analysis Division, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The U.S. Environmental Protection Agency (EPA) is proposing effluent limitations guidelines and standards for promulgation at 40 *CFR* Part 430 for the Pulp, Paper, and Paperboard industrial category to control the discharge of pollutants into surface waters of the United States. This compendium of test procedures (methods) supports the proposal. The purpose of publishing this compendium is to provide a single source of methods that are unique to the proposed rule. These methods must be used for filing permit applications and for compliance monitoring under the National Pollutant Discharge Elimination System (NPDES) program.

The methods included in this compendium are updated versions of methods included in the June 1991 compendium titled "Analytical Methods for the Pulp and Paper Industry Study." Results from this and other EPA studies were used to revise certain specifications in the methods contained in this compendium.

This compendium includes only those methods that are unique to the pulp and paper rule-making. Other methods allowed under the proposed rule have been promulgated at 40 *CFR* Part 136.

Questions concerning the methods in this compendium should be directed to:

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Contents

Cross-Reference

Analytes shown in ***bold italic type*** are regulated under the proposed effluent limitations guidelines for the Pulp, Paper, and Paperboard Industry found at 40 *CFR* Part 430.

Analytes	*CAS No.*	*Applicable Method(s)*
Adsorbable Organic Halides (AOX)	***59473-04-0***	***1650***
4-Chlorocatechol	2138-22-9	1653
4-Chloroguaiacol	16766-30-6	1653
4-Chlorophenol	106-48-9	1653
2-Chlorosyringaldehyde	76341-69-0	1653
5-Chlorovanillin	19463-48-0	1653
6-Chlorovanillin	18268-76-3	1653
Color	***M-002[1]***	***NCASI 253***
3,4-Dichlorocatechol	3978-67-4	1653
3,6-Dichlorocatechol	3938-16-7	1653
4,5-Dichlorocatechol	3428-24-8	1653
3,4-Dichloroguaiacol	77102-94-4	1653
4,5-Dichloroguaiacol	2460-49-3	1653
4,6-Dichloroguaiacol	16766-31-7	1653
2,4-Dichlorophenol	120-83-2	1653
2,6-Dichlorophenol	87-65-0	1653
2,6-Dichlorosyringaldehyde	76330-06-8	1653
5,6-Dichlorovanillin	18268-69-4	1653
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	35822-46-9	1613
Heptachlorodibenzo-*p*-dioxins	37871-00-4	1613
1,2,3,4,6,7,8-Heptachlorodibenzofuran	67562-39-4	1613
1,2,3,4,7,8,9-Heptachlorodibenzofuran	55673-89-7	1613
Heptachlorodibenzofurans	38998-75-3	1613
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	39227-28-6	1613
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	57653-85-7	1613
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	19408-74-3	1613
Hexachlorodibenzo-*p*-dioxins	34465-46-8	1613
1,2,3,4,7,8-Hexachlorodibenzofuran	70648-26-9	1613
1,2,3,6,7,8-Hexachlorodibenzofuran	57117-44-9	1613
1,2,3,7,8,9-Hexachlorodibenzofuran	72918-21-9	1613
2,3,4,6,7,8-Hexachlorodibenzofuran	60851-34-5	1613
Hexachlorodibenzofurans (Total)	55684-94-1	1613
Octachlorodibenzo-*p*-dioxin	3268-87-9	1613
Octachlorodibenzofuran	39001-02-0	1613
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	40321-76-4	1613

[1]This is a synthetic CAS number taken from EPA's Environmental Monitoring Methods Index (EMMI).

Cross-Reference (cont.)

Analytes	*CAS No.*	*Applicable Method(s)*
Pentachlorodibenzo-p-dioxins	36088-22-9	1613
1,2,3,7,8-Pentachlorodibenzofuran	57117-41-6	1613
2,3,4,7,8-Pentachlorodibenzofuran	57117-31-4	1613
Pentachlorodibenzofurans (Total)	30402-15-4	1613
Pentachlorophenol	***87-86-5***	***1653***
Tetrachlorocatechol	***1198-55-6***	***1653***
Tetrachlorodibenzo-*p*-dioxins	41903-57-5	1613
2,3,7,8-Tetrachlorodibenzo-p-dioxin	***1746-01-6***	***1613***
2,3,7,8-Tetrachlorodibenzofuran	***1207-31-9***	***1613***
Tetrachlorodibenzofurans	55722-27-5	1613
Tetrachloroguaiacol	***2539-17-5***	***1653***
2,3,4,6-Tetrachlorophenol	***58-90-2***	***1653***
3,4,5-Trichlorocatechol	***56961-20-7***	***1653***
3,4,6-Trichlorocatechol	***32139-72-3***	***1653***
3,4,5-Trichloroguaiacol	***57057-83-7***	***1653***
3,4,6-Trichloroguaiacol	***60712-44-9***	***1653***
4,5,6-Trichloroguaiacol	***2668-24-8***	***1653***
2,4,5-Trichlorophenol	***95-95-4***	***1653***
2,4,6-Trichlorophenol	***88-006-2***	***1653***
Trichlorosyringol	***2539-26-6***	***1653***

Method 1613

Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

Revision A

October 1993

Method 1613

Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

1. SCOPE AND APPLICATION

1.1 This method is designed to meet the survey requirements of the USEPA Engineering and Analysis Division (EAD). The method is used to determine the tetra- through octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans associated with the Clean Water Act (as amended in 1987); the Resource Conservation and Recovery Act (as amended in 1986); and the Comprehensive Environmental Response, Compensation, and Liability Act (as amended in 1986); and other dioxin and furan compounds amenable to high resolution capillary column gas chromatography high-resolution mass spectrometry (HRGC/HRMS). Specificity is provided for determination of the 17 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF).

1.2 The method is based on a compilation of EPA, industry, commercial laboratory, and academic methods (References 1 through 6).

1.3 The tetra- through octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans listed in Table 1 may be determined in waters, soils, sludges, and other matrices by this method.

1.4 The detection limits of the method are usually dependent on the level of interferences rather than instrumental limitations. The levels in Table 2 typify the minimum quantities that can be quantitatively determined in environmental samples using the method.

1.5 The GC/MS portions of the method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

2.1 Stable isotopically labeled analogs of 15 of the PCDDs and PCDFs are added to each sample prior to extraction. Samples containing coarse solids are prepared for extraction by grinding or homogenization. Water samples are filtered and then extracted with methylene chloride using separatory funnel procedures; the particulates from the water samples, soils, and other finely divided solids are extracted using a combined Soxhlet extraction/Dean-Stark azeotropic distillation (Reference 7). Prior to cleanup and analysis, the extracts of the filtered water and the particulates are combined.

2.2 After extraction, $^{37}Cl_4$-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. Sample cleanups may include back-extraction with acid and/or base, gel permeation, alumina, silica gel, and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of the 2,3,7,8- isomers or other specific isomers or congeners.

2.3 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, two internal standards are added to each extract, and a 1-μL aliquot of the extract is injected into

the gas chromatograph. The analytes are separated by the GC and detected by a high-resolution ($\geq$10,000) mass spectrometer. Two exact masses (m/z's) are monitored for each analyte.

2.4 Dioxins and furans are identified by comparing GC retention times and the ion abundance ratios of the m/z's with the corresponding retention-time ranges of authentic standards and the theoretical ion-abundance ratios of the exact m/z's. Isomers and congeners are identified when the retention times and ion abundance ratios agree within predefined limits. By using a GC column or columns capable of resolving the 2,3,7,8-substituted isomers from all other tetra-isomers, the 2,3,7,8-substituted isomers are identified when the retention-time and m/z abundance ratios agree within predefined limits of the retention times and exact m/z ratios of authentic standards.

2.5 Quantitative analysis is performed by GC/MS using selected ion current profile (SICP) areas, in one of two ways.

2.5.1 For the 15 2,3,7,8-substituted isomers for which labeled analogs are available (see Table 1), the GC/MS system is calibrated and the compound concentration is determined using an isotope dilution technique. Although a labeled analog of the octachlorinated dibenzofuran (OCDF) is available, using high-resolution mass spectrometry it produces an m/z that may interfere with the identification and quantitation of the unlabeled octachlorinated dibenzo-*p*-dioxin (OCDD). Therefore, this labeled analog has not been included in the calibration standards, and the unlabeled OCDF is quantitated against the labeled OCDD. Because the labeled analog of 1,2,3,7,8,9-HxCDD is used as an internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the unlabeled compound by strict isotope dilution procedures. Therefore, the unlabeled 1,2,3,7,8,9-HxCDD is quantitated using the average of the responses of the labeled analogs of the other two 2,3,7,8-substituted HxCDDs (i.e., 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD). As a result, the concentration of the unlabeled 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDDs.

2.5.2 For non-2,3,7,8-substituted isomers and the total concentrations of all isomers within a level of chlorination (i.e., total TCDD), concentrations are determined using response factors from the calibration of labeled analogs at the same level of chlorination.

2.6 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems.

3. CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms (References 8 and 9). Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

3.2 Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

3.2.1 Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with PTFE stopcocks, must be disassembled prior to detergent washing.

3.2.2 After detergent washing, glassware should be immediately rinsed, first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, then acetone, and then methylene chloride.

3.2.3 Do not bake reusable glassware in an oven as a routine part of cleaning. Baking may be warranted after particularly dirty samples are encountered but should be minimized, as repeated baking of glassware may cause active sites on the glass surface that will irreversibly adsorb PCDDs/PCDFs.

3.2.4 Immediately prior to use, Soxhlet extraction glassware should be pre-extracted with toluene for approximately 3 hours (see Section 11.2.3). Separatory funnels should be shaken with methylene chloride/toluene (80/20 mixture) for 2 minutes, drained, and then shaken with pure methylene chloride for 2 minutes.

3.3 All materials used in the analysis shall be demonstrated to be free from interferences by running reference matrix blanks initially and with each sample set (samples started through the extraction process on a given 12-hour shift, to a maximum of 20 samples). The reference matrix blank must simulate, as closely as possible, the sample matrix under test. Reagent water (Section 6.6.1) is used to simulate water samples; playground sand (Section 6.6.2) or white quartz sand (Section 6.3.2) can be used to simulate soils; filter paper (Section 6.6.3) is used to simulate papers and similar materials; other materials (Section 6.6.4) can be used to simulate other matrices.

3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the PCDDs and PCDFs. The most frequently encountered interferences are chlorinated biphenyls, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, polynuclear aromatics, and pesticides. Because very low levels of PCDDs and PCDFs are measured by this method, the elimination of interferences is essential. The cleanup steps given in Section 12 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PCDDs and PCDFs at the levels shown in Table 2.

3.5 Each piece of reusable glassware should be numbered in such a fashion that the laboratory can associate all reusable glassware with the processing of a particular sample. This will assist the laboratory in (1) tracking down possible sources of contamination for individual samples, (2) identifying glassware associated with highly contaminated samples that may require extra cleaning, and (3) determining when glassware should be discarded.

4. SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

4.1.1 The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. It is soluble in water to approximately 200 ppt and in organic solvents to 0.14%. On the basis of the available toxicological and physical properties of 2,3,7,8-TCDD, all of the PCDDs and PCDFs should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

4.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator shall be worn when high concentrations are handled.

4.2 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 data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 10 through 13. The references and bibliography at the end of Reference 13 are particularly comprehensive in dealing with the general subject of laboratory safety.

4.3 The PCDDs and PCDFs and samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. The PCDDs and PCDFs are extremely toxic to laboratory animals. Each laboratory must develop a strict safety program for handling the PCDDs and PCDFs. The following practices are recommended (References 2 and 14).

4.3.1 Facility: When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

4.3.2 Protective equipment: Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be utilized. During analytical operations which may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection equipment (preferably full face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands. When handling samples suspected or known to contain high concentrations of the PCDDs or PCDFs, an additional set of gloves can also be worn beneath the latex gloves.

4.3.3 Training: Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

4.3.4 Personal hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

4.3.5 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic absorbent paper on bench tops.

4.3.6 Effluent vapors: The effluents of sample splitters for the gas chromatograph and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

4.3.7 Waste Handling and Disposal.

4.3.7.1 Handling: Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

4.3.7.2 Disposal.

4.3.7.2.1 The PCDDs and PCDFs decompose above 800°C. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels which are capable of handling extremely toxic wastes.

4.3.7.2.2 Liquid or soluble waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength shorter than 290 nm for several days. (Use F40 BL lamps or equivalent.) Analyze liquid wastes and dispose of the solutions when the PCDDs and PCDFs can no longer be detected.

4.3.8 Decontamination.

4.3.8.1 Personal decontamination: Use any mild soap with plenty of scrubbing action.

4.3.8.2 Glassware, tools, and surfaces: Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. If glassware is first rinsed with solvent, then the dish water may be disposed of in the sewer. Given the cost of disposal, it is prudent to minimize solvent wastes.

4.3.9 Laundry: Clothing known to be contaminated should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

4.3.10 Wipe tests: A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by GC can achieve a limit of detection of 0.1 μg per wipe. Less than 0.1 μg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 μg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of

the equipment or work space, and indicates that unacceptable work practices have been employed.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps.

5.1.1.1 Liquid samples (waters, sludges and similar materials containing 5% solids or less): Sample bottle, amber glass, 1.1 L minimum, with screw cap.

5.1.1.2 Solid samples (soils, sediments, sludges, paper pulps, filter cake, compost, and similar materials that contain more than 5% solids): Sample bottle, wide mouth, amber glass, 500-mL minimum.

5.1.1.3 If amber bottles are not available, samples shall be protected from light.

5.1.1.4 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.1.5 Cleaning.

5.1.1.5.1 Bottles are detergent-water washed, then solvent-rinsed before use.

5.1.1.5.2 Liners are detergent-water washed, then rinsed with reagent water (Section 6.6.1) and then solvent, and baked at approximately 200°C for a minimum of 1 hour prior to use.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Glass or PTFE tubing only shall be used. 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 rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for glassware cleaning: Laboratory sink with overhead fume hood.

5.3 Equipment for sample preparation.

5.3.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

5.3.2 Glove box (optional).

5.3.3 Tissue homogenizer: VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

5.3.4 Meat grinder: Hobart, or equivalent, with 3- to 5-mm holes in inner plate.

5.3.5 Equipment for determining percent moisture.

5.3.5.1 Oven: Capable of maintaining a temperature of 110 ±5°C.

5.3.5.2 Dessicator.

5.3.6 Balances.

5.3.6.1 Analytical: Capable of weighing 0.1 mg.

5.3.6.2 Top loading: Capable of weighing 10 mg.

5.4 Extraction apparatus.

5.4.1 Water samples.

5.4.1.1 pH meter, with combination glass electrode.

5.4.1.2 pH paper, wide range (Hydrion Papers, or equivalent).

5.4.1.3 Graduated cylinder, 1 L capacity.

5.4.1.4 1 L filtration flasks with side arm, for use in vacuum-filtration of water samples.

5.4.1.5 Separatory funnels: 250-, 500-, and 2000-mL, with PTFE stopcocks.

5.4.2 Soxhlet/Dean-Stark (SDS) extractor (see Figure 1).

5.4.2.1 Soxhlet: 50 mm ID, 200-mL capacity with 500-mL flask (Cal-Glass LG-6900, or equivalent, except substitute 500-mL round-bottom flask for 300-mL flat-bottom flask).

5.4.2.2 Thimble: 43 × 123 to fit Soxhlet (Cal-Glass LG-6901-122, or equivalent).

5.4.2.3 Moisture trap: Dean Stark or Barret with PTFE stopcock, to fit Soxhlet.

5.4.2.4 Heating mantle: Hemispherical, to fit 500-mL round-bottom flask (Cal-Glass LG-8801-112, or equivalent).

5.4.2.5 Variable transformer: Powerstat (or equivalent), 110-volt, 10-amp.

5.4.3 Beakers: 400- to 500-mL.

5.4.4 Spatulas: Stainless steel.

5.5 Filtration apparatus.

5.5.1 Pyrex glass wool: Solvent-extracted by SDS for 3 hours minimum.

NOTE: Baking glass wool may cause active sites that will irreversibly adsorb PCDDs/PCDFs.

5.5.2 Glass funnel: 125- to 250-mL.

5.5.3 Glass fiber filter paper (Whatman GF/D, or equivalent).

5.5.4 Drying column: 15 to 20 mm ID Pyrex chromatographic column equipped with coarse-glass frit or glass-wool plug.

5.5.5 Buchner funnel, 15 cm.

5.5.6 Glass fiber filter paper for above.

5.5.7 Pressure filtration apparatus: Millipore YT30 142 HW, or equivalent.

5.6 Centrifuge apparatus.

5.6.1 Centrifuge: Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.

5.6.2 Centrifuge bottles: 500-mL, with screw-caps, to fit centrifuge.

5.6.3 Centrifuge tubes: 12- to 15-mL, with screw-caps, to fit centrifuge.

5.7 Cleanup apparatus.

5.7.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).

5.7.1.1 Column: 600 to 700 mm long × 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

5.7.1.2 Syringe: 10-mL, with Luer fitting.

5.7.1.3 Syringe filter holder, stainless steel, and glass fiber or PTFE filters (Gelman 4310, or equivalent).

5.7.1.4 UV detectors: 254-nm, preparative or semi-prep flow cell (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 μL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

5.7.2 Reverse-phase high-performance liquid chromatograph.

5.7.2.1 Column oven and detector: Perkin-Elmer Model LC-65T (or equivalent) operated at 0.02 AUFS at 235 nm.

5.7.2.2 Injector: Rheodyne 7120 (or equivalent) with 50-μL sample loop.

5.7.2.3 Column: Two 6.2 mm × 250 mm Zorbax-ODS columns in series (DuPont Instruments Division, Wilmington, DE, or equivalent), operated at 50°C with 2.0 mL/min methanol isocratic effluent.

5.7.2.4 Pump: Altex 110A (or equivalent).

5.7.3 Pipets.

5.7.3.1 Disposable, Pasteur, 150 mm long × 5 mm ID (Fisher Scientific 13-678-6A, or equivalent).

5.7.3.2 Disposable, serological, 10-mL (6 mm ID).

5.7.4 Chromatographic columns.

5.7.4.1 150 mm long × 8 mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250-mL reservoir.

5.7.4.2 200 mm long × 15 mm ID, with coarse-glass frit or glass-wool plug and 250-mL reservoir.

5.7.5 Oven: For storage of adsorbents, capable of maintaining a temperature of 130°C (±5°C).

5.8 Concentration apparatus.

5.8.1 Rotary evaporator: Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath.

5.8.1.1 A vacuum source is required for use of the rotary evaporator. It must be equipped with a shutoff valve at the evaporator and, preferably, have a vacuum gauge.

5.8.1.2 A recirculating water pump and chiller are recommended, as use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

5.8.1.3 Round-bottom flasks: 100-mL and 500-mL or larger, with ground-glass fitting compatible with the rotary evaporator.

5.8.2 Kuderna-Danish (K-D).

5.8.2.1 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.8.2.2 Evaporation flask: 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).

5.8.2.3 Snyder column: Three-ball macro (Kontes K-503000-0232, or equivalent).

5.8.2.4 Boiling chips.

5.8.2.4.1 Glass or silicon carbide: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for a minimum of one hour.

5.8.2.4.2 Teflon (optional): Extracted with methylene chloride.

5.8.2.5 Water bath: Heated, with concentric ring cover, capable of maintaining a temperature within ±2°C, installed in a fume hood.

5.8.3 Nitrogen evaporation apparatus: Equipped with water bath controlled at 35 to 40°C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

5.8.4 Sample vials: Amber glass, 2- to 5-mL with Teflon-lined screw-cap.

5.9 Gas chromatograph: Shall have splitless or on-column injection port for capillary column, temperature program with isothermal hold, and shall meet all of the performance specifications in Section 7.

5.9.1 GC column for PCDDs and PCDFs and for isomer specificity for 2,3,7,8-TCDD: 60 m (±5 m) long × 0.32 mm (±0.02 mm) ID; 0.25 μm 5% phenyl, 94% methyl, 1% vinyl silicone bonded-phase fused-silica capillary column (J & W DB-5, or equivalent).

5.9.2 GC column for isomer specificity for 2,3,7,8-TCDF: 30 m (±5 m) long × 0.32 mm (±0.02 mm) ID; 0.25 μm bonded-phase fused-silica capillary column (J & W DB-225, or equivalent).

5.10 Mass spectrometer: 28 to 40 eV electron impact ionization, shall be capable of repetitively selectively monitoring 12 exact m/z's minimum at high resolution (≥10,000) during a period of approximately 1 second, and shall meet all of the performance specifications in Section 7.

5.11 GC/MS interface: The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beams.

5.12 Data system: Capable of collecting, recording, and storing MS data.

6. REAGENTS AND STANDARDS

6.1 pH adjustment and back-extraction.

6.1.1 Potassium hydroxide: Dissolve 20 g reagent grade KOH in 100 mL reagent water.

6.1.2 Sulfuric acid: Reagent grade (specific gravity 1.84).

6.1.3 Sodium chloride: Reagent grade, prepare a 5% (w/v) solution in reagent water.

6.2 Solution drying and evaporation.

6.2.1 Solution drying: Sodium sulfate, reagent grade, granular anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400°C for 1 hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

6.2.2 Prepurified nitrogen.

6.3 Extraction.

6.3.1 Solvents: Acetone, toluene, cyclohexane, hexane, methanol, methylene chloride, and nonane; distilled in glass, pesticide quality, lot-certified to be free of interferences.

6.3.2 White quartz sand, 60/70 mesh: For Soxhlet/Dean-Stark extraction (Aldrich Chemical, Cat. No. 27-437-9, or equivalent). Bake at 450°C for a minimum of 4 hours.

6.4 GPC calibration solution: Prepare a solution containing 300 mg/mL corn oil, 15 mg/mL bis(2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.

6.5 Adsorbents for sample cleanup.

6.5.1 Silica gel.

6.5.1.1 Activated silica gel: Bio-Sil A, 100-200 mesh (Bio-Rad 131-1340, or equivalent), rinsed with methylene chloride, baked at 180°C for a minimum of 1 hour, cooled in a dessicator, and stored in a precleaned glass bottle with screw-cap that prevents moisture from entering.

6.5.1.2 Acid silica gel (30% w/w): Thoroughly mix 44.0 g of concentrated sulfuric acid with 100.0 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with PTFE-lined cap.

6.5.1.3 Basic silica gel: Thoroughly mix 30 g of 1N sodium hydroxide with 100 g of activated silica gel in a clean container. Break up aggregates with a

stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with PTFE-lined cap.

6.5.2 Alumina: Either one of two types of alumina, acid or basic, may be used in the cleanup of sample extracts, provided that the laboratory can meet the performance specifications for the recovery of labeled compounds described in Section 8.3. The same type of alumina must be used for all samples, including those used to demonstrate initial precision and accuracy (Section 8.2) and ongoing precision and accuracy (Section 14.5).

6.5.2.1 Acid alumina: Bio-Rad Laboratories 132-1340 Acid Alumina AG 4 (or equivalent). Activate by heating to 130°C for a minimum of 12 hours.

6.5.2.2 Basic alumina: Bio-Rad Laboratories 132-1240 Basic Alumina AG 10 (or equivalent). Activate by heating to 600°C for a minimum of 24 hours. Alternatively, activate by heating alumina in a tube furnace at 650 to 700°C under an air flow of approximately 400 cc/min. Do not heat over 700°C, as this can lead to reduced capacity for retaining the analytes. Store at 130°C in a covered flask. Use within five days of baking.

6.5.3 AX-21/Celite.

6.5.3.1 Activated carbon: AX-21 (Anderson Development Company, Adrian, MI, or equivalent). Prewash with methanol and dry *in vacuo* at 110°C.

6.5.3.2 Celite 545: (Supelco 2-0199, or equivalent).

6.5.3.3 Thoroughly mix 5.35 g AX-21 and 62.0 g Celite 545 to produce a 7.9% w/w mixture. Activate the mixture at 130°C for a minimum of 6 hours. Store in a dessicator.

6.6 Reference matrices.

6.6.1 Reagent water: Water in which the PCDDs and PCDFs and interfering compounds are not detected by this method.

6.6.2 High-solids reference matrix: Playground sand or similar material in which the PCDDs and PCDFs and interfering compounds are not detected by this method. May be prepared by extraction with methylene chloride and/or baking at 450°C for a minimum of 4 hours.

6.6.3 Filter paper: Gelman type A (or equivalent) glass fiber filter paper in which the PCDDs and PCDFs and interfering compounds are not detected by this method. Cut the paper to simulate the surface area of the paper sample being tested.

6.6.4 Other matrices: This method may be verified on any matrix by performing the tests given in Section 8.2. Ideally, the matrix should be free of the PCDDs and PCDFs, but in no case shall the background level of the PCDDs and PCDFs in the reference matrix exceed three times the minimum levels given in Table 2. If low background levels of the PCDDs and PCDFs are present in the reference matrix, the spike level of the analytes used in Section 8.2 should be increased to provide a spike-to-background ratio in the range of 1:1 to 5:1 (Reference 15).

6.7 Standard solutions: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition.

If the chemical purity is 98% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at room temperature in screw-capped vials with PTFE-lined caps. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. If solvent loss has occurred, the solution should be replaced.

6.8 Stock solutions.

6.8.1 Preparation: Prepare in nonane per the steps below or purchase as dilute solutions (Cambridge Isotope Laboratories, Woburn, MA, or equivalent). Observe the safety precautions in Section 4, and the recommendation in Section 4.1.2.

6.8.2 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 1 to 2 mg of 2,3,7,8-TCDD to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with nonane. After the TCDD is completely dissolved, transfer the solution to a clean 15-mL vial with PTFE-lined cap.

6.8.3 Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards. Reference standards that can be used to determine the accuracy of calibration standards are available from Cambridge Isotope Laboratories and may be available from other vendors.

6.9 Secondary standard: Using stock solutions (Section 6.8), prepare secondary standard solutions containing the compounds and concentrations shown in Table 4 in nonane.

6.10 Labeled-compound stock standard: From stock standard solutions prepared as above, or from purchased mixtures, prepare this standard to contain the labeled compounds at the concentrations shown in Table 4 in nonane. This solution is diluted with acetone prior to use (Section 10.3.2).

6.11 Cleanup standard: Prepare $^{37}Cl_4$-2,3,7,8-TCDD at the concentration shown in Table 4 in nonane.

6.12 Internal standard: Prepare at the concentration shown in Table 4 in nonane.

6.13 Calibration standards (CS1 through CS5): Combine the solutions in Sections 6.9, 6.10, 6.11, and 6.12 to produce the five calibration solutions shown in Table 4 in nonane. These solutions permit the relative response (labeled to unlabeled) and response factor to be measured as a function of concentration. The CS3 standard is used for calibration verification (VER).

6.14 Precision and recovery standard (PAR): Used for determination of initial (Section 8.2) and ongoing (Section 14.5) precision and accuracy. This solution contains the analytes and labeled compounds at the concentrations listed in Table 4 in nonane. This solution is diluted with acetone prior to use (Sections 10.3.4 and 10.4.4).

6.15 GC retention time window defining solutions: Used to define the beginning and ending retention times for the dioxin and furan isomers.

6.15.1 DB-5 column window defining standards, Cambridge Isotope Laboratories ED-1732-A (dioxins) and ED-1731-A (furans), or equivalent, containing the compounds listed in Table 5.

6.16 Isomer specificity test standards: Used to demonstrate isomer specificity of the GC columns employed for the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzodibenzofuran.

6.16.1 Standards for the DB-5 column: Cambridge Isotope Laboratories ED-908, ED-908-C, or ED-935, or equivalent, containing the compounds listed in Table 5 at concentrations of approximately 10 μg/mL of each compound.

6.16.2 Standards for the DB-225 column: Cambridge Isotope Laboratories EF-937 or EF-938, or equivalent, containing the compounds listed in Table 5 at concentrations of approximately 10 μg/mL of each compound.

6.17 Stability of solutions: Standard solutions used for quantitative purposes (Sections 6.9 through 6.14) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area at the quantitation m/z remains within ±15% of the area obtained in the initial analysis of the standard. Any standards failing to meet this criterion should be assayed against reference standards, as in Section 6.8.3., before further use.

7. CALIBRATION

7.1 Assemble the GC/MS and establish the operating conditions necessary to meet the relative retention time specifications in Table 2.

7.1.1 The following GC operating conditions may be used for guidance and adjusted as needed to meet the relative retention time specifications in Table 2:

Injector temperature:	270°C
Interface temperature:	290°C
Initial temperature:	200°C
Initial time:	2 minutes
Temperature program:	200 to 220°C, at 5°C/min
	220°C for 16 minutes
	220 to 235°C, at 5°C/min
	235°C for 7 minutes
	235 to 330°C, at 5°C/min

NOTE: All portions of the column which connect the GC to the ion source shall remain at the interface temperature specified above during analysis, to preclude condensation of less volatile compounds.

7.1.2 Mass spectrometer (MS) resolution: Obtain a selected ion current profile (SICP) of each analyte in Table 4 at the two exact masses specified in Table 3 and at ≥10,000 resolving power by injecting an authentic standard of the PCDDs and PCDFs either singly or as part of a mixture in which there is no interference between closely eluted components.

7.1.2.1 The analysis time for PCDDs and PCDFs may exceed the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass)

can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory. A lock-mass ion from the reference compound (PFK) is used for tuning the mass spectrometer. The lock-mass ion is dependent on the masses of the ions monitored within each descriptor, as shown in Table 3. The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless of the descriptor number) does not exceed 10% of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in time lost in cleaning the source.

7.1.2.2 Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). For each descriptor (Table 3), monitor and record the resolution and exact mass of three to five reference peaks covering the mass range of the descriptor. The resolution must be greater than or equal to 10,000. The deviation between the exact mass and the theoretical mass (Table 3) for each ion monitored must be less than 5 ppm.

7.2 Ion abundance ratios, minimum levels, signal-to-noise ratios, and absolute retention times: Inject an aliquot of the CS1 calibration solution (Table 4) using the GC conditions from Section 7.1.

7.2.1 Measure the SICP areas for each analyte and compute the ion abundance ratios specified in Table 3A. Compare the computed ratio to the theoretical ratio given in Table 3A.

7.2.1.1 The groups of m/z's to be monitored are shown in Table 3. Each group or descriptor shall be monitored in succession as a function of GC retention time to ensure that all PCDDs and PCDFs are detected. Additional m/z's may be monitored in each descriptor, and the m/z's may be divided among more than the five descriptors listed in Table 3, provided that the laboratory is able to monitor the m/z's of all the PCDDs/PCDFs that may elute from the GC in a given retention-time window.

7.2.1.2 The mass spectrometer shall be operated in a mass-drift correction mode, using perfluorokerosene (PFK) to provide lock masses. The lock-mass for each group of m/z's is shown in Table 3. Each lock-mass shall be monitored and shall not vary by more than $\pm 20\%$ throughout its respective retention time window. Variations of the lock-mass by more than 20% indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Reinjection of another

aliquot of the sample extract will not resolve the problem. Additional cleanup of the extract may be required to remove the interferences.

7.2.2 All PCDDs and PCDFs in the CS1 standard (both labeled and unlabeled) shall be within the QC limits in Table 3A for their respective ion abundance ratios; otherwise, the mass spectrometer shall be adjusted and this test repeated until the m/z ratios fall within the limits specified. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 7.1) prior to repeat of the test.

7.2.3 Verify that the HRGC/HRMS instrument meets the minimum levels in Table 2. The peaks representing both unlabeled and labeled analytes in the CS1 calibration standard must have signal-to-noise ratios (S/N) greater than or equal to 10.0. Otherwise, the mass spectrometer shall be adjusted and this test repeated until the minimum levels in Table 2 are met.

7.2.4 The absolute retention time of $^{13}C_{12}$-1,2,3,4-TCDD (Section 6.12) shall exceed 25.0 minutes on the DB-5 column, and the retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 15.0 minutes on the DB-225 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the above-stated minimum retention time criteria are met.

7.3 Retention-time windows: Analyze the window defining mixtures (Section 6.15) using the optimized temperature program in Section 13 (Figures 2A through 2D). Table 5 gives the elution order (first/last) of the compound pairs.

7.4 Isomer specificity

7.4.1 Analyze the isomer specificity test standards (Section 6.16) using the procedure in Section 13.

7.4.2 Compute the percent valley between the GC peaks that elute most closely to the 2,3,7,8-TCDD and TCDF isomers, on their respective columns, per Figure 3.

7.4.3 Verify that the height of the valley between the most closely eluted isomers and the 2,3,7,8-substituted isomers is less than 25% (computed as 100 x/y in Figure 3). If the valley exceeds 25%, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (Section 7.2 through 7.5).

7.5 Calibration with isotope dilution: Isotope dilution is used for the 15 2,3,7,8-substituted PCDDs and PCDFs with labeled compounds added to the samples prior to extraction, and with minor modifications for 1,2,3,7,8,9-HxCDD and OCDF (see Section 16.1). The reference compound for each unlabeled compound is shown in Table 6.

7.5.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (RR) (unlabeled to labeled) vs. concentration in standard solutions is plotted or computed using a linear regression. Relative response is determined according to the procedures described below. A minimum of five data points are employed for calibration.

7.5.2 The relative response of each unlabeled PCDD/PCDF and its labeled analog is determined using the area responses of both the primary and secondary m/z's specified in Table 3, for each calibration standard, as follows:

$$RR = \frac{(A_n^1 + A_n^2)\ C_l}{(A_l^1 + A_l^2)\ C_n}$$

Where:

A_n^1 *and* A_n^2 = *The areas of the primary and secondary m/z's for the unlabeled compound.*

A_l^1 *and* A_l^2 = *The areas of the primary and secondary m/z's for the labeled compound.*

C_l = *The concentration of the labeled compound in the calibration standard.*

C_n = *The concentration of the unlabeled compound in the calibration standard.*

7.5.3 To calibrate the analytical system by isotope dilution, inject a 1.0-μL aliquot of calibration standards CS1 through CS5 (Section 6.13 and Table 4) using the procedure in Section 13 and the conditions in Table 2. Compute the relative response (RR) at each concentration.

7.5.4 Linearity: If the relative response for any compound is constant (less than 20% coefficient of variation) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

7.6 Calibration by internal standard: The internal standard method is applied to determination of non-2,3,7,8-substituted compounds having no labeled analog in this method, and to measurement of labeled compounds for intralaboratory statistics (Sections 8.4 and 14.5.4).

7.6.1 Response factors: Calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{(A_s^1 + A_s^2)\ C_{is}}{(A_{is}^1 + A_{is}^2)\ C_s}$$

Where:

A_s^1 *and* A_s^2 = *The areas of the primary and secondary m/z's for the compound to be calibrated.*

A_{is}^1 *and* A_{is}^2 = *The areas of the primary and secondary m/z's for the internal standard.*

C_{is} = *The concentration of the internal standard (Section 6.12 and Table 4).*

C_s = *The concentration of the compound in the calibration standard.*

NOTE: There is only one m/z for $^{37}Cl_4$-2,3,7,8-TCDD. See Table 3.

7.6.2 To calibrate the analytical system by internal standard, inject a 1.0 μL aliquot of calibration standards CS1 through CS5 (Section 6.13 and Table 4) using the procedure in Section 13 and the conditions in Table 2. Compute the response factor (RF) at each concentration.

7.6.3 Linearity: If the response factor (RF) for any compound is constant (less than 35% coefficient of variation) over the five-point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point range.

7.7 Combined calibration: By using calibration solutions (Section 6.13 and Table 4) containing the unlabeled and labeled compounds and the internal standards, a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods. These curves are verified each shift (Section 14.3) by analyzing the calibration verification standard (VER, Table 4). Recalibration is required if any of the calibration verification criteria (Section 14.3.5) cannot be met.

7.8 Data storage: MS data shall be collected, recorded, and stored.

7.8.1 Data acquisition: The signal at each exact m/z shall be collected repetitively throughout the monitoring period and stored on a mass storage device.

7.8.2 Response factors and multipoint calibrations: The data system shall be used to record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibration curves. Computations of relative standard deviation (coefficient of variation) shall be used to test calibration linearity. Statistics on initial performance (Section 8.2) and ongoing performance (Section 14.5) shall be computed and maintained, either on the instrument data system, or on a separate computer system.

8. QUALITY ASSURANCE/QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 16). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method. If the method is to be applied routinely to samples containing high-solids with very little moisture (e.g., soils, filter cake, compost) or to an alternate matrix, the high-solids reference matrix (Section 6.6.2) or the alternate matrix (Section 6.6.4) is substituted for the reagent water matrix (Section 6.6.1) in all performance tests.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided that all performance specifications are met. Each time a modification is made to the method, the analyst is required to repeat the procedures in Section 8.2 to demonstrate method performance.

8.1.3 Analyses of blanks are required to demonstrate freedom from contamination (Section 3.2). The procedures and criteria for analysis of a blank are described in Section 8.5.

8.1.4 The laboratory shall spike all samples with labeled compounds to monitor method performance. This test is described in Section 8.3. When results of these spikes

indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits. Procedures for dilutions are given in Section 16.5.

8.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the ongoing precision and recovery aliquot that the analytical system is in control. These procedures are described in Sections 14.1 through 14.5.

8.1.6 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.2 Initial precision and recovery (IPR): To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.

8.2.1 For low solids (aqueous) samples, extract, concentrate, and analyze four 1-L aliquots of reagent water spiked with the diluted precision and recovery standard (PAR) (Sections 6.14 and 10.3.4) according to the procedures in Sections 10 through 13. For an alternative sample matrix, four aliquots of the alternative matrix are used. All sample processing steps that are to be used for processing samples, including preparation (Section 10), extraction (Section 11), and cleanup (Section 12), shall be included in this test.

8.2.2 Using results of the set of four analyses, compute the average concentration (X) of the extracts in ng/mL and the standard deviation of the concentration (s) in ng/mL for each compound, by isotope dilution for PCDDs and PCDFs with a labeled analog, and by internal standard for labeled compounds.

8.2.3 For each unlabeled and labeled compound, compare s and X with the corresponding limits for initial precision and accuracy in Table 7. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for accuracy, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 8.2). The concentration limits in Table 7 for labeled compounds are based on the requirement that the recovery of each labeled compound be in the range of 25 to 150%.

8.3 The laboratory shall spike all samples and QC aliquots with the diluted labeled compound spiking solution (Sections 6.10 and 10.3.2) to assess method performance on the sample matrix.

8.3.1 Analyze each sample according to the procedures in Sections 10 through 13.

8.3.2 Compute the percent recovery of the labeled compounds and the cleanup standard using the internal standard method (Section 16.2).

8.3.3 The recovery of each labeled compound must be within 25 to 150%. If the recovery of any compound falls outside of these limits, method performance is unacceptable for that compound in that sample. To overcome such difficulties, water samples are diluted and smaller amounts of soils, sludges, sediments, and other matrices are reanalyzed per Section 17.

8.4 Method accuracy for samples shall be assessed and records shall be maintained.

8.4.1 After the analysis of five samples of a given matrix type (water, soil, sludge, pulp, etc.) for which the labeled compound spiking standards pass the tests in Section 8.3, compute the average percent recovery (R) and the standard deviation of the percent recovery (S_R) for the labeled compounds only. Express the accuracy assessment as a percent recovery interval from $R - 2S_R$ to $R + 2S_R$ for each matrix. For example, if $R = 90\%$ and $S_R = 10\%$ for five analyses of pulp, the accuracy interval is expressed as 70 to 110%.

8.4.2 Update the accuracy assessment for each compound in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).

8.5 Blanks: Reference matrix blanks are analyzed to demonstrate freedom from contamination (Section 3.2).

8.5.1 Prepare, extract, clean up, and concentrate a method blank with each sample set (samples of the same matrix started through the extraction process on the same 12-hour shift, to a maximum of 20 samples). The matrix for the method blank shall be similar to sample matrix for the set, e.g., a 1-L reagent water blank (Section 6.6.1), high-solids reference matrix blank (Section 6.6.2), paper matrix blank (Section 6.6.3) or alternative reference matrix blank (Section 6.6.4). Analyze the blank immediately after analysis of the precision and recovery standard (Section 14.5) to demonstrate freedom from contamination.

8.5.2 If any of the 2,3,7,8-substituted PCDDs or PCDFs (Table 1) is found in the blank at greater than the minimum level (Table 2), assuming a response factor of 1 relative to the $^{13}C_{12}$-1,2,3,4-TCDD internal standard for compounds not listed in Table 1, analysis of samples is halted until the source of contamination is eliminated and a new blank is analyzed that shows no evidence of contamination at this level.

NOTE: All samples associated with a contaminated method blank must be re-extracted and reanalyzed before the results may be reported for regulatory compliance purposes.

8.6 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 14.3), and for initial (Section 8.2) and ongoing (Section 14.5) precision and recovery should be identical, so that the most precise results will be obtained. A GC/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of PCDDs and PCDFs by this method.

8.7 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Collect samples in amber glass containers following conventional sampling practices (Reference 17). Aqueous samples which flow freely are collected in refrigerated bottles using

automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.

9.2 Maintain samples at 0 to 4°C in the dark from the time of collection until extraction. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 18).

9.3 Perform sample analysis within 40 days of extraction.

10. SAMPLE PREPARATION

The sample preparation process involves modifying the physical form of the sample so that the PCDDs and PCDFs can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Table 8 lists the phase(s) and quantity extracted for various sample matrices. Samples containing a solid phase and samples containing particle sizes larger than 1 mm require preparation prior to extraction. Because PCDDs/PCDFs are strongly associated with particulates, the preparation of aqueous samples is dependent on the solids content of the sample. Aqueous samples containing 1% solids or less are extracted in a separatory funnel. A smaller sample aliquot is used for aqueous samples containing more than 1% solids. For samples expected or known to contain high levels of the PCDDs and/or PCDFs, the smallest sample size representative of the entire sample should be used, and the sample extract should be diluted, if necessary, per Section 16.5.

10.1 Determine percent solids.

10.1.1 Weigh 5 to 10 g of sample (to three significant figures) into a tared beaker.

NOTE: This aliquot is used only for determining the solids content of the sample, not for analysis of PCDDs/PCDFs.

10.1.2 Dry overnight a minimum of 12 hours at 110°C (±5°C), and cool in a dessicator.

10.1.3 Calculate percent solids as follows:

$$\% \text{ solids} = \frac{\text{weight of sample aliquot after drying}}{\text{weight of sample aliquot before drying}} \times 100$$

10.2 Determine particle size.

10.2.1 Spread the dried sample from Section 10.1.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

10.2.2 Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1 mm or less prior to extraction.

10.3 Preparation of aqueous samples containing 1% solids or less: The extraction procedure for aqueous samples containing less than or equal to 1% solids involves filtering the sample, extracting the particulate phase and the filtrate separately, and combining the extracts for

analysis. The aqueous portion is extracted by shaking with methylene chloride in a separatory funnel. The particulate material is extracted using the SDS procedure.

10.3.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample in the bottle on a top loading balance to ± 1 g.

10.3.2 Dilute a sufficient volume of the labeled compound stock solution by a factor of 50 with acetone to prepare the labeled compound spiking solution. Each sample requires 1.0 mL of the diluted solution, but no more solution should be prepared than can be used in one day. Spike 1.0 mL of the diluted solution into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1 to 2 hours, with occasional shaking.

10.3.3 For each sample or sample set (to a maximum of 20 samples) to be extracted during the same 12-hour shift, place two 1.0-L aliquots of reagent water in clean 2-L separatory flasks.

10.3.4 Spike 1.0 mL of the diluted labeled-compound spiking standard (Section 6.10) into one reagent water aliquot. This aliquot will serve as the blank. Dilute 10 μL of the precision and recovery standard (Section 6.14) to 2.0 mL with acetone. Spike 1.0 mL of the diluted precision and recovery standard into the remaining reagent water aliquot. This aliquot will serve as the PAR (Section 14.5). Spike 1.0 mL of the diluted labeled compound spiking standard (Section 6.10) into the PAR aliquot as well.

10.3.5 Assemble a Buchner funnel on top of a clean 1-L filtration flask. Apply a vacuum to the flask, and pour the entire contents of the sample bottle through a glass fiber filter (Section 5.5.4) in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particulates.

10.3.6 Rinse the sample bottle twice with 5 mL of reagent water to transfer any remaining particulates onto the filter.

10.3.7 Rinse the any particulates off the sides of the Buchner funnel with small quantities of reagent water.

10.3.8 Weigh the empty sample bottle on a top-loading balance to ± 1 g. Determine the weight of the sample by difference. Do not discard the bottle at this point.

10.3.9 Extract the filtrates using the procedures in Section 11.1.1.

10.3.10 Extract the particulates using the procedures in Section 11.2.

10.4 Preparation of samples containing greater than 1% solids.

10.4.1 Weigh a well-mixed aliquot of each sample (of the same matrix type) sufficient to provide 10 g of dry solids (based on the solids determination in 10.1.3) into a clean beaker or glass jar.

10.4.2 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 10.3.2) into the sample aliquot(s).

10.4.3 For each sample or sample set (to a maximum of 20 samples) to be extracted during the same 12-hour shift, weigh two 10-g aliquots of the appropriate reference matrix (Section 6.6) into clean beakers or glass jars.

10.4.4 Spike 1.0 mL of the diluted labeled compound spiking solution into one reference matrix aliquot. This aliquot will serve as the blank. Spike 1.0 mL of the diluted precision and recovery standard into the remaining reference matrix aliquot. This aliquot will serve as the PAR (Section 14.5). Spike 1.0 mL of the diluted labeled compound spiking solution into the PAR aliquot as well.

10.4.5 Stir or tumble and equilibrate the aliquots for 1 to 2 hours.

10.4.6 Extract the aliquots using the procedures in Section 11.

10.5 Multiphase samples

10.5.1 Pressure filter the sample, blank, and PAR aliquots through Whatman GF/D glass fiber filter paper. If necessary, centrifuge these aliquots for 30 minutes at greater than 5000 rpm prior to filtration.

10.5.2 Discard any aqueous phase (if present). Remove any non-aqueous liquid (if present) and reserve for recombination with the extract of the solid phase (Section 11.2.5). Prepare the filter papers of the sample and QC aliquots for particle size reduction and blending (Section 10.6).

10.6 Sample grinding, homogenization, or blending: Samples with particle sizes greater than 1 mm (as determined by Section 10.2.2) are subjected to grinding, homogenization, or blending. The method of reducing particle size to less than 1 mm is matrix-dependent. In general, hard particles can be reduced by grinding with a mortar and pestle. Softer particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or by blending.

10.6.1 Each size-reducing preparation procedure on each matrix shall be verified by running the tests in Section 8.2 before the procedure is employed routinely.

10.6.2 The grinding, homogenization, or blending procedures shall be carried out in a glove box or fume hood to prevent particles from contaminating the work environment.

10.6.3 Grinding: Tissue samples, certain papers and pulps, slurries, and amorphous solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots from Section 10.4.5 or 10.5.2 in a clean grinder. Do not allow the sample temperature to exceed 50 °C. Grind the blank and reference matrix aliquots using a clean grinder.

10.6.4 Homogenization or blending: Particles that are not ground effectively, or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the sample, blank, and PAR aliquots from Section 10.4.5, 10.5.2, or 10.6.3.

10.6.5 Extract the aliquots using the procedures in Section 11.

11. EXTRACTION AND CONCENTRATION

11.1 Extraction of filtrates: Extract the aqueous samples, blanks, and IPR/OPR aliquots according to the following procedures.

11.1.1 Pour the filtered aqueous sample from the filtration flask into a 2-L separatory funnel. Rinse the flask twice with 5 mL of reagent water and add these rinses to the

separatory funnel. Add 60 mL methylene chloride to the sample bottle (Section 10.3.8), seal, and shake 60 seconds to rinse the inner surface.

11.1.2 Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting. 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, employ mechanical techniques to complete the phase separation (see note below). Drain the methylene chloride extract into a solvent-rinsed glass funnel approximately one-half full of clean sodium sulfate. Set up the glass funnel so that it will drain directly into a solvent-rinsed 500-mL K-D concentrator fitted with a 10-mL concentrator tube.

NOTE: *The formation of emulsions can be expected in any solvent extraction procedure. If an emulsion forms, the analyst must take steps to break the emulsion before proceeding. Mechanical means of breaking the emulsion include, but are not limited to, use of a glass stirring rod, filtration through glass wool, and other techniques. For emulsions that resist these techniques, centrifugation may be required.*

Experience with aqueous samples high in dissolved organic materials (e.g., paper mill effluents) has shown that acidification of the sample prior to extraction may reduce the formation of emulsions. Paper industry methods suggest that the addition of up to 400 mL of ethanol to a 1-L effluent sample may also reduce emulsion formation. However, studies by the Environmental Protection Agency to date suggest that the effect may be a result of the dilution of the sample, and that the addition of reagent water may serve the same function. Mechanical techniques may still be necessary to complete the phase separation. If either acidification or addition of ethanol is utilized, the laboratory must perform the startup tests described in Section 8.2 using the same techniques.

11.1.3 Extract the water sample two more times using 60 mL of fresh methylene chloride each time. Drain each extract through the funnel containing the sodium sulfate into the K-D concentrator. After the third extraction, rinse the separatory funnel with at least 20 mL of fresh methylene chloride, and drain this rinse through the sodium sulfate into the concentrator. Repeat this rinse at least twice.

11.1.4 The extract of the filtrate must be concentrated before it is combined with the extract of the particulates for further cleanup. Add one or two clean boiling chips to the receiver and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of hexane through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

11.1.5 Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

11.1.6 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent 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 hexane. A 5-mL syringe is recommended for this operation.

11.1.7 The concentrated extracts of the filtrate and the particulates are combined using the procedures in Section 11.2.13.

11.2 Soxhlet/Dean-Stark extraction of solids: Extract the solid samples, particulates, blanks, and IPR/OPR aliquots using the following procedure.

11.2.1 Charge a clean extraction thimble with 5.0 g of 100/200 mesh silica (Section 6.5.1.1) and 100 g of quartz sand (Section 6.3.2).

NOTE: Do not disturb the silica layer throughout the extraction process.

11.2.2 Place the thimble in a clean extractor. Place 30 to 40 mL of toluene in the receiver and 200 to 250 mL of toluene in the flask.

11.2.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 to 2 drops of toluene per second will fall from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours.

11.2.4 After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with toluene and allow to air dry.

11.2.5 Load the wet sample from Sections 10.4.5, 10.5.2, 10.6.3, or 10.6.4, and any non-aqueous liquid from Section 10.5.2 into the thimble and manually mix into the sand layer with a clean metal spatula carefully breaking up any large lumps of sample. If the material to be extracted is the particulate matter from the filtration of an aqueous sample, add the filter paper to the thimble also.

11.2.6 Reassemble the pre-extracted SDS apparatus and add a fresh charge of toluene to the receiver and reflux flask.

11.2.7 Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Check the apparatus for foaming frequently during the first 2 hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

11.2.8 Drain the water from the receiver at 1 to 2 hours and 8 to 9 hours, or sooner if the receiver fills with water. Reflux the sample for a total of 16 to 24 hours. Cool and disassemble the apparatus. Record the total volume of water collected.

11.2.9 Remove the distilling flask. Drain the water from the Dean-Stark receiver and add any toluene in the receiver to the extract in the flask.

11.2.10 For solid samples, the extract must be concentrated to approximately 10 mL prior to back extraction. For the particulates filtered from an aqueous sample, the extract must be concentrated prior to combining with the extract of the filtrate. Therefore, add one or two clean boiling chips to the round-bottom flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of toluene through the top. Place the round-bottom flask in a heating mantle and apply heat as required to complete the concentration in 15 to 20 minutes. At the proper rate of

distillation, the balls of the column will actively chatter but the chambers will not flood.

11.2.11 When the liquid has reached an apparent volume of 10 mL, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column.

11.2.12 If the extract is from a solid sample, not the particulates from an aqueous sample, transfer the concentrated extract to a 250-mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with back-extraction per Section 11.3.

11.2.13 If the extract is from the particulates from an aqueous sample, it must be combined with the concentrated extract of the filtrate (Section 11.1.7) prior to back extraction. Assemble the glass funnel filled approximately one-half full with sodium sulfate from Section 11.1.2 such that the funnel will drain into the K-D concentrator from Section 11.1.7 containing the concentrated methylene chloride extract of the filtrate. Pour the concentrated toluene extract of the particulates through the sodium sulfate into the K-D concentrator. Rinse the round-bottom flask with three 15- to 20- mL volumes of hexane, and pour each rinse through the sodium sulfate into the K-D concentrator. Add one or two fresh boiling chips to the receiver and attach the three-ball macro Snyder column to the K-D concentrator. Prewet the column by adding approximately 1 mL of hexane to the top of the column. Concentrate the combined extract to approximately 10 mL (the volume of the toluene). Remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column. Transfer the contents of the K-D concentrator to a prerinsed 250-mL separatory funnel. Rinse the flask and lower joint with three 5-mL volumes of hexane, and add each rinse to the separatory funnel. Proceed with back extraction per Section 11.3.

11.3 Back extraction with base and acid.

11.3.1 Spike 1.0 mL of the cleanup standard (Section 6.11) into the separatory funnels containing the sample and QC extracts (Section 11.2.12 or 11.2.13).

11.3.2 Partition the extract against 50 mL of potassium hydroxide solution (Section 6.1.1). Shake for 2 minutes with periodic venting into a hood. Remove and discard the aqueous layer. Repeat the base washing until no color is visible in the aqueous layer, to a maximum of four washings. Minimize contact time between the extract and the base to prevent degradation of the PCDDs and PCDFs. Stronger potassium hydroxide solutions may be employed for back extraction, provided that the laboratory meets the specifications for labeled compound recovery and demonstrates acceptable performance using the procedures in Section 8.2.

11.3.3 Partition the extract against 50 mL of sodium chloride solution (Section 6.1.3) in the same way as with base. Discard the aqueous layer.

11.3.4 Partition the extract against 50 mL of sulfuric acid (Section 6.1.2) in the same way as with base. Repeat the acid washing until no color is visible in the aqueous layer, to a maximum of four washings.

11.3.5 Repeat the partitioning against sodium chloride solution and discard the aqueous layer.

11.3.6 Pour each extract through a drying column containing 7 to 10 cm of anhydrous sodium sulfate. Rinse the separatory funnel with 30 to 50 mL of toluene and pour through the drying column. Collect each extract in a 500-mL round-bottom flask. Concentrate and clean up the samples and QC aliquots per Sections 11.4 and 12.

11.4 Macro-concentration: Concentrate the extracts in separate 100-mL round bottom flasks on a rotary evaporator.

11.4.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, preclean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for contamination check if necessary. Between samples, three 2- to 3-mL aliquots of toluene should be rinsed down the feed tube into a waste beaker.

11.4.2 Attach the round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

11.4.3 Lower the flask into the water bath and adjust the speed of rotation and the temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

NOTE: If the rate of concentration is too fast, analyte loss may occur.

11.4.4 When the liquid in the concentration flask has reached an apparent volume of 2 mL, remove the flask from the water bath and stop the rotation. Slowly and carefully, admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 mL of hexane.

11.4.5 Transfer the extract to a vial using three 2- to 3-mL rinses of hexane. Proceed with micro-concentration and solvent exchange.

11.5 Micro-concentration and solvent exchange.

11.5.1 Toluene extracts to be subjected to GPC or HPLC cleanup are exchanged into methylene chloride. Extracts that are to be cleaned up using silica gel, alumina, and/or AX-21/Celite are exchanged into hexane.

11.5.2 Transfer the vial containing the sample extract to a nitrogen evaporation device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

NOTE: A large vortex in the solvent may cause analyte loss.

11.5.3 Lower the vial into a 45°C water bath and continue concentrating.

11.5.4 When the volume of the liquid is approximately 100 μL, add 2 to 3 mL of the desired solvent (methylene chloride or hexane) and continue concentration to approximately 100 μL. Repeat the addition of solvent and concentrate once more.

11.5.5 If the extract is to be cleaned up by GPC or HPLC, adjust the volume of the extract to 5.0 mL with methylene chloride. Proceed with GPC cleanup (Section 12.2).

11.5.6 If the extract is to be cleaned up by column chromatography (alumina, silica gel, AX-21/Celite), bring the final volume to 1.0 mL with hexane. Proceed with column cleanups (Sections 12.3 through 12.5).

11.5.7 For extracts to be concentrated for injection into the GC/MS: Quantitatively transfer the extract to a 0.3-mL conical vial for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 μL. Add 10 μL of nonane to the vial, and evaporate the solvent to the level of the nonane.

11.5.8 Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/MS analysis.

12. EXTRACT CLEANUP

12.1 Cleanup may not be necessary for relatively clean samples (e.g., treated effluents, groundwater, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. Before using a cleanup procedure, the analyst must demonstrate that the requirements of Section 8.2 can be met using the cleanup procedure.

12.1.1 Gel permeation chromatography (Section 12.2) removes many high molecular weight interferences that cause GC column performance to degrade. It may be used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids).

12.1.2 Acid, neutral, and basic silica gel, and alumina (Sections 12.3 and 12.4) are used to remove nonpolar and polar interferences.

12.1.3 AX-21/Celite (Section 12.5) is used to remove nonpolar interferences.

12.1.4 HPLC (Section 12.6) is used to provide specificity for the 2,3,7,8-substituted and other PCDD and PCDF isomers.

12.2 Gel permeation chromatography (GPC).

12.2.1 Column packing.

12.2.1.1 Place 70 to 75 g of SX-3 Bio-beads in a 400- to 500-mL beaker.

12.2.1.2 Cover the beads with methylene chloride and allow to swell overnight (a minimum of 12 hours).

12.2.1.3 Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.

12.2.1.4 After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.

12.2.2 Column calibration.

12.2.2.1 Load 5 mL of the calibration solution (Section 6.4) into the sample loop.

12.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethyl hexyl) phthalate, pentachlorophenol, perylene, and sulfur.

12.2.2.3 Set the "dump time" to allow >85% removal of the corn oil and >85% collection of the phthalate.

12.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

12.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

12.2.3 Extract cleanup: GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5 mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into aliquots for GPC and the aliquots are combined after elution from the column. The residue content of the extract may be obtained gravimetrically by evaporating the solvent from a 50-μL aliquot.

12.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0-mL extract onto the column.

12.2.3.2 Elute the extract using the calibration data determined in Section 12.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

12.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

12.2.3.4 If a particularly dirty extract is encountered, a 5.0-mL methylene chloride blank shall be run through the system to check for carry-over.

12.2.3.5 Concentrate the eluate per Sections 11.2.1, 11.2.2, and 11.3.1 or 11.3.2 for further cleanup or for injection into the GC/MS.

12.3 Silica gel cleanup.

12.3.1 Place a glass-wool plug in a 15-mm-ID chromatography column. Pack the column in the following order (bottom to top): 1 g silica gel (Section 6.5.1.1), 4 g basic silica gel (Section 6.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 6.5.1.2), 2 g silica gel. Tap the column to settle the adsorbents.

12.3.2 Prerinse the column with 50 to 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

12.3.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

12.3.4 Rinse the receiver twice with 1-mL portions of hexane and apply separately to the column. Elute the PCDDs/PCDFs with 100 mL hexane and collect the eluate.

12.3.5 Concentrate the eluate per Section 11.4 or 11.5 for further cleanup or for injection into the HPLC or GC/MS.

12.3.6 For extracts of samples known to contain large quantities of other organic compounds (such as paper mill effluents), it may be advisable to increase the capacity of the silica gel column. This may be accomplished by increasing the strengths of the acid and basic silica gels. The acid silica gel (Section 6.5.1.2) may be increased in strength to as much as 44% w/w (7.9 g sulfuric acid added to 10 g silica gel). The basic silica gel (Section 6.5.1.3) may be increased in strength to as much as 33% w/w (50 mL 1N NaOH added to 100 g silica gel).

NOTE: The use of stronger acid silica gel (44% w/w) may lead to charring of organic compounds in some extracts. The charred material may retain some of the analytes and lead to lower recoveries of PCDDs/PCDFs. Increasing the strengths of the acid and basic silica gel may also require different volumes of hexane than those specified above, to elute the analytes off the column. Therefore, the performance of the method after such modifications must be verified by the procedures in Section 8.2.

12.4 Alumina cleanup.

12.4.1 Place a glass-wool plug in a 15-mm-ID chromatography column.

12.4.2 If using acid alumina, pack the column by adding 6 g acid alumina (Section 6.5.2.1). If using basic alumina, substitute 6 g basic alumina (Section 6.5.2.2). Tap the column to settle the adsorbents.

12.4.3 Prerinse the column with 50 to 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the alumina.

12.4.4 Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

12.4.5 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the alumina.

12.4.6 Rinse the receiver twice with 1-mL portions of hexane and apply separately to the column. Elute the interfering compounds with 100 mL hexane and discard the eluate.

12.4.7 The choice of eluting solvents will depend on the choice of alumina (acid or basic) made in Section 12.4.2.

12.4.7.1 If using acid alumina, elute the PCDDs and PCDFs from the column with 20 mL methylene chloride:hexane (20:80 v/v). Collect the eluate.

12.4.7.2 If using basic alumina, elute the PCDDs and PCDFs from the column with 20 mL methylene chloride:hexane (50:50 v/v). Collect the eluate.

12.4.8 Concentrate the eluate per Section 11.4 or 11.5 for further cleanup or for injection into the HPLC or GC/MS.

12.5 AX-21/Celite.

12.5.1 Cut both ends from a 10-mL disposable serological pipet to produce a 10-cm column. Fire-polish both ends and flare both ends if desired. Insert a glass-wool plug at one end, then pack the column with 1 g of the activated AX-21/Celite to form an

adsorbent bed 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

12.5.2 Prerinse the column with 5 mL of toluene followed by 2 mL methylene chloride:methanol:toluene (15:4:1 v/v), 1 mL methylene chloride:cyclohexane (1:1 v/v), and 5 mL hexane. If the flow rate of eluate exceeds 0.5 mL/min, discard the column.

12.5.3 When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1-mL portions of hexane and apply separately to the column. Apply 2 mL of hexane to complete the transfer.

12.5.4 Elute the interfering compounds with 2 mL of hexane, 2 mL of methylene chloride:cyclohexane (1:1 v/v), and 2 mL of methylene chloride:methanol:toluene (15:4:1 v/v). Discard the eluate.

12.5.5 Invert the column and elute the PCDDs and PCDFs with 20 mL of toluene. If carbon particles are present in the eluate, filter through glass fiber filter paper.

12.5.6 Concentrate the eluate per Section 11.4 or 11.5 for further cleanup or for injection into the HPLC or GC/MS.

12.6 HPLC (adapted from Reference 6).

12.6.1 Column calibration.

12.6.1.1 Prepare a calibration standard containing the 2,3,7,8-substituted isomers and/or other isomers of interest at a concentration of approximately 500 pg/μL in methylene chloride.

12.6.1.2 Inject 30 μL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetra- through octa-isomers.

12.6.1.3 Establish the collect time for the tetra-isomers and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of methylene chloride, including a minimum of five 50-μL injections while the detector is monitored, to ensure that residual PCDDs and PCDFs are removed from the system.

12.6.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the PCDDs and PCDFs from the calibration standard (Section 12.6.1.1) is 75 to 125% compared to the calibration (Section 12.6.1.2). If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated system.

12.6.2 Extract cleanup: HPLC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 30 μL of extract. If the extract cannot be concentrated to less than 30 μL, it is split into fractions and the fractions are combined after elution from the column.

12.6.2.1 Rinse the sides of the vial twice with 30 μL of methylene chloride and reduce to 30 μL with the evaporation apparatus.

12.6.2.2 Inject the 30 μL extract into the HPLC.

12.6.2.3 Elute the extract using the calibration data determined in Section 12.6.1. Collect the fraction(s) in a clean 20-mL concentrator tube containing 5 mL of hexane:acetone (1:1 v/v).

12.6.2.4 If an extract containing greater than 100 ng/mL of total PCDD or PCDF is encountered, a 30-μL methylene chloride blank shall be run through the system to check for carry-over.

12.6.2.5 Concentrate the eluate per Section 11.5 for injection into the GC/MS.

13. HRGC/HRMS ANALYSIS

13.1 Establish the operating conditions given in Section 7.1.

13.2 Add 10 μL of the internal standard solution (Section 6.12) to the sample extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be reanalyzed and evaporation has occurred, do *not* add more instrument internal standard solution. Rather, bring the extract back to its previous volume (e.g., 19 μL) with pure nonane only.

13.3 Inject 1.0 μL of the concentrated extract containing the internal standard solution, using on-column or splitless injection. Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Stop data collection after the OCDD and OCDF have eluted. Return the column to the initial temperature for analysis of the next extract or standard.

14. SYSTEM AND LABORATORY PERFORMANCE

14.1 At the beginning of each 12-hour shift during which analyses are performed, GC/MS system performance and calibration are verified for all unlabeled and labeled compounds. For these tests, analysis of the CS3 calibration verification (VER) standard (Section 6.13 and Table 4) and the isomer specificity test standards (Section 6.16 and Table 5) shall be used to verify all performance criteria. Adjustment and/or recalibration (Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.

14.2 MS resolution: A static resolving power of at least 10,000 (10% valley definition) must be demonstrated at appropriate masses before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour shift according to procedures in Section 7.1.2. Corrective actions must be implemented whenever the resolving power does not meet the requirement.

14.3 Calibration verification.

14.3.1 Inject the VER standard using the procedure in Section 13.

14.3.2 The m/z abundance ratios for all PCDDs and PCDFs shall be within the limits in Table 3A; otherwise, the mass spectrometer shall be adjusted until the m/z abundance ratios fall within the limits specified, and the verification test (Section 14.4.1) shall be repeated. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 7.1.2) prior to repeat of the verification test.

14.3.3 The peaks representing each unlabeled and labeled compound in the VER standard must be present with S/N of at least 10; otherwise, the mass spectrometer shall be adjusted and the verification test (Section 14.4.1) repeated.

14.3.4 Compute the concentration of each unlabeled compound by isotope dilution (Section 7.5) for those compounds that have labeled analogs (Table 1). Compute the concentration of the labeled compounds by the internal standard method. These concentrations are computed based on the calibration data in Section 7.

14.3.5 For each compound, compare the concentration with the calibration verification limit in Table 7. If all compounds meet the acceptance criteria, calibration has been verified. If, however, any compound fails, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (Section 14.2) and verification (Section 14.3.1) tests, or recalibrate (Section 7).

14.4 Retention times and GC resolution.

14.4.1 Retention times.

14.4.1.1 Absolute: The absolute retention times of the $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD GCMS internal standards shall be within ± 15 seconds of the retention times obtained during calibration (Section 7.2.4).

14.4.1.2 Relative: The relative retention times of unlabeled and labeled PCDDs and PCDFs shall be within the limits given in Table 2.

14.4.2 GC resolution.

14.4.2.1 Inject the isomer specificity standards (Section 6.16) on their respective columns.

14.4.2.2 The valley height between 2,3,7,8-TCDD and the other tetra-dioxin isomers at m/z 319.8965, and between 2,3,7,8-TCDF and the other tetra-furan isomers at m/z 303.9016 shall not exceed 25% on their respective columns (Figure 3).

14.4.3 If the absolute retention time of any compound is not within the limits specified or the 2,3,7,8-isomers are not resolved, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (Section 14.3.1) or recalibrate (Section 7).

14.5 Ongoing precision and recovery.

14.5.1 Analyze the extract of the ongoing precision and recovery (OPR) aliquot (Section 10.3.4 or 10.4.4) prior to analysis of samples from the same set.

14.5.2 Compute the concentration of each PCDD and PCDF by isotope dilution for those compounds that have labeled analogs (Section 7.5). Compute the concentration of each labeled compound by the internal standard method.

14.5.3 For each unlabeled and labeled compound, compare the concentration with the limits for ongoing accuracy in Table 7. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, the

extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-extract the sample set (Section 10) and repeat the ongoing precision and recovery test (Section 14.5). The concentration limits in Table 7 for labeled compounds are based on the requirement that the recovery of each labeled compound be in the range of 25 to 150%.

14.5.4 Add results which pass the specifications in Section 14.5.3 to initial and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each PCDD and PCDF in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (S_R). Express the accuracy as a recovery interval from $R - 2S_R$ to $R + 2S_R$. For example, if $R = 95\%$ and $S_R = 5\%$, the accuracy is 85 to 105%.

14.6 Blank analysis: Analyze a method blank extracted along with the sample set to be analyzed. The blank is analyzed immediately following the analysis of the OPR aliquot to demonstrate freedom from contamination and freedom from carryover from the OPR analysis. The results of the blank analysis must meet the specifications in Section 8.5 before ample analyses may proceed.

15. QUALITATIVE DETERMINATION

For a gas chromatographic peak to be identified as a PCDD or PCDF (either an unlabeled or a labeled compound), it must meet *all* of the criteria in Sections 15.1 through 15.4.

15.1 The signals for the two exact m/z's being monitored (Table 3) must be present and must maximize within ±2 seconds of one another.

15.2 The signal-to-noise ratio (S/N) of each of the two exact m/z's must be greater than or equal to 2.5 for a sample extract, and greater than or equal to 10 for a calibration standard (see Sections 7.2.3 and 14.3.3.

15.3 The ratio of the integrated ion currents of both the exact m/z's monitored must be within the limits in Table 3A.

15.4 The relative retention time of the peaks representing an unlabeled 2,3,7,8-substituted PCDD or PCDF must be within the limits given in Table 2. The retention time of peaks representing non-2,3,7,8-substituted PCDDs or PCDFs must be within the retention-time windows established in Section 7.3.

15.5 Confirmatory analysis: Isomer specificity for all of the 2,3,7,8-substituted analytes cannot be attained by analysis on the DB-5 (or equivalent) GC column alone. The lack of specificity is of greatest concern for the unlabeled 2,3,7,8-TCDF. Therefore, any sample in which 2,3,7,8-TCDF is identified by analysis on a DB-5 (or equivalent) GC column must have a confirmatory analysis performed on a DB-225, SP-2330, or equivalent GC column. The operating conditions in Section 7.1.1 may be adjusted for analyses on the second GC column, but the GC/MS must meet the mass resolution and calibration specifications in Section 7.

15.7 If any gas chromatographic peak that represents a labeled analog does not meet all of the identification criteria in Sections 15.1 through 15.4 on the second GC column, then the results may not be reported for regulatory compliance purposes and a new aliquot of the sample must be extracted and analyzed.

16. QUANTITATIVE DETERMINATION

16.1 Isotope dilution: By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the unlabeled compound can be made because the unlabeled compound and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography. Relative response (RR) values are used in conjunction with the initial calibration data described in Section 7.5 to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A_n^1 + A_n^2)\ C_{is}}{(A_l^1 + A_l^2)\ RR}$$

where:
C_{ex} = *The concentration of the unlabeled compound in the extract and the other terms are as defined in Sections* 7.5.2 *and* 7.6.1.

16.1.1 Because of a potential interference, the labeled analog of OCDF is not added to the sample. Therefore, this unlabeled analyte is quantitated against the labeled OCDD. As a result, the concentration of unlabeled OCDF is corrected for the recovery of the labeled OCDD. In instances where OCDD and OCDF behave differently during sample extraction, concentration, and cleanup procedures, this may decrease the accuracy of the OCDF results. However, given the low toxicity of this compound relative to the other dioxins and furans, the potential decrease in accuracy is not considered significant.

16.1.2 Because the labeled analog of 1,2,3,7,8,9-HxCDD is used as an internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the unlabeled compound by strict isotope dilution procedures. Therefore, the unlabeled 1,2,3,7,8,9-HxCDD is quantitated using the average of the responses of the labeled analogs of the other two 2,3,7,8-substituted HxCDD's, 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD. As a result, the concentration of the unlabeled 1,2,3,7,8,9-HxCDD is corrected for the average recovery of the other two HxCDD's.

16.1.3 Any peaks representing non-2,3,7,8-substituted dioxins or furans are quantitated using an average of the response factors from all of the labeled 2,3,7,8- isomers in the same level of chlorination.

16.2 Internal standard: Compute the concentrations of the ^{13}C-labeled analogs and the ^{37}C-labeled cleanup standard in the extract using the response factors determined from the initial calibration data (Section 7.6) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A_s^1 + A_s^2)\ C_{is}}{(A_{is}^1 + A_{is}^2)\ RF}$$

where:
C_{ex} = *The concentration of the compound in the extract. The other terms are defined in Section* 7.6.1

NOTE: There is only one m/z for the ^{37}Cl-labeled standard.

16.3 The concentration of the unlabeled compound in the solid phase of the sample is computed using the concentration of the compound in the extract and the weight of the solids (Section 10), as follows:

$$\textit{Concentration in solid (ng/kg)} = \frac{(C_{ex} \times V_{ex})}{W_s}$$

where:
V_{ex} = *The extract volume in mL.*
W_s = *The sample weight (dry weight) in kg.*

16.4 The concentration of the unlabeled compound in the aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted (Section 10.3), as follows:

$$\textit{Concentration in aqueous phase (pg/L)} = \frac{(C_{ex} \times V_{ex})}{V_s}$$

where:
V_{ex} = *The extract volume in mL.*
V_s = *The sample volume in liters.*

16.5 If the SICP areas at the quantitation m/z's for any compound exceed the calibration range of the system, a smaller sample aliquot is extracted.

16.5.1 For aqueous samples containing 1% solids or less, dilute 100 mL, 10 mL, etc., of sample to 1 L with reagent water and extract per Section 11.

16.5.2 For samples containing greater than 1% solids, extract an amount of sample equal to 1/10, 1/100, etc., of the amount determined in Section 10.1.3. Extract per Section 10.4.

16.5.3 If a smaller sample size will not be representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/μL in the extract, and analyze an aliquot of this diluted extract by the internal standard method.

16.6 Results are reported to three significant figures for the unlabeled and labeled isomers found in all standards, blanks, and samples. For aqueous samples, the units are pg/L; for samples containing greater than 1% solids (soils, sediments, filter cake, compost), the units are ng/Kg based on the dry weight of the sample.

16.6.1 Results for samples which have been diluted are reported at the least dilute level at which the areas at the quantitation m/z's are within the calibration range (Section 16.5).

16.6.2 For unlabeled compounds having a labeled analog, results are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range

(Section 16.5) and the labeled compound recovery is within the normal range for the method (Section 17.3).

16.6.3 Additionally, if requested, the total concentration of all isomers in an individual level of chlorination (i.e., total TCDD, total TCDF, total PeCDD, etc.) may be reported by summing the concentrations of all isomers identified in that level of chlorination, including both 2,3,7,8-substituted and non-2,3,7,8-substituted isomers.

17. ANALYSIS OF COMPLEX SAMPLES

17.1 Some samples may contain high levels (>10 ng/L; >1000 ng/Kg) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts will not concentrate to 10 μL (Section 11); others may overload the GC column and/or mass spectrometer.

17.2 Analyze a smaller aliquot of the sample (Section 16.5) when the extract will not concentrate to 20 μL after all cleanup procedures have been exhausted.

17.3 Recovery of labeled compounds: In most samples, recoveries of the labeled compounds will be similar to those from reagent water or from the alternate matrix (Section 6.6). If recovery of any of the labeled compounds is outside of the 25 to 150% range, a diluted sample (Section 16.5) shall be analyzed. If the recoveries of any of the labeled compounds in the diluted sample are outside of the limits (per the criteria above), then the calibration verification standard (Section 14.3) shall be analyzed and calibration verified (Section 14.3.5). If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed. If the calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, then the method does not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes.

18. METHOD PERFORMANCE

The performance specifications in this method are based on the analyses of more than 400 samples, representing matrices from at least five industrial categories. These specifications will be updated periodically as more data are received, and each time the procedures in the method are revised.

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Table 1. Polychlorinated Dibenzo-*p*-dioxins and Furans Determined by Isotope Dilution and Internal Standard High-Resolution Gas Chromatography (HRGC)/High-Resolution Mass Spectrometry (HRMS)

PCDDs/PCDFs[1]	*CAS Registry*	*Labeled Analog*	*CAS Registry*
2,3,7,8-TCDD	1746-01-6	$^{13}C_{12}$-2,3,7,8-TCDD $^{37}Cl_4$-2,3,7,8-TCDD	76523-40-5 85508-50-5
Total TCDD	41903-57-5	--	--
2,3,7,8-TCDF	51207-31-9	$^{13}C_{12}$-2,3,7,8-TCDF	89059-46-1
Total-TCDF	55722-27-5	--	--
1,2,3,7,8-PeCDD	40321-76-4	$^{13}C_{12}$-1,2,3,7,8-PeCDD	109719-79-1
Total-PeCDD	36088-22-9	--	--
1,2,3,7,8-PeCDF	57117-41-6	$^{13}C_{12}$-1,2,3,7,8-PeCDF	109719-77-9
2,3,4,7,8-PeCDF	57117-31-4	$^{13}C_{12}$-2,3,4,7,8-PeCDF	116843-02-8
Total-PeCDF	30402-15-4	--	--
1,2,3,4,7,8-HxCDD	39227-28-6	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD[2]	109719-82-6
Total-HxCDD	34465-46-8	--	--
1,2,3,4,7,8-HxCDF	70648-26-9	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	116843-05-1
Total-HxCDF	55684-94-1	--	--
1,2,3,4,6,7,8-HpCDD	35822-46-9	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	109719-83-7
Total-HpCDD	37871-00-4	--	--
1,2,3,4,6,7,8-HpCDF	67562-39-4	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	109719-84-8
1,2,3,4,7,8,9-HpCDF	55673-89-7	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	109719-94-0
Total-HpCDF	38998-75-3	--	--
OCDD	3268-87-9	$^{13}C_{12}$-OCDD	114423-97-1
OCDF	39001-02-0	none	--

1. Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans

TCDD	=	Tetrachlorodibenzo-*p*-dioxin	TCDF	=	Tetrachlorodibenzofuran
PeCDD	=	Pentachlorodibenzo-*p*-dioxin	PeCDF	=	Pentachlorodibenzofuran
HxCDD	=	Hexachlorodibenzo-*p*-dioxin	HxCDF	=	Hexachlorodibenzofuran
HpCDD	=	Heptachlorodibenzo-*p*-dioxin	HpCDF	=	Heptachlorodibenzofuran
OCDD	=	Octachlorodibenzo-*p*-dioxin	OCDF	=	Octachlorodibenzofuran

2. Labeled analog is used as an injection internal standard and therefore is not used for quantitation of the unlabeled compound.

Table 2. Retention Times and Minimum Levels for PCDDs and PCDFs

Compound	Retention Time Reference	Relative Retention Time	Minimum Level[1] Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/µL; ppb)
Compounds using $^{13}C_{12}$-1,2,3,4-TCDD as the injection internal standard					
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0.999–1.001	10	1	0.5
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	0.999–1.001	10	1	0.5
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999–1.001	50	5	2.5
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999–1.001	50	5	2.5
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0.999–1.001	50	5	2.5
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD	0.931–0.994			
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0.993–1.036			
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1.002–1.013			
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.091–1.371			
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1.123–1.408			
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1.134–1.428			
Compounds using $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as the injection internal standard					
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0.999–1.001	50	5	2.5
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0.999–1.001	50	5	2.5
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0.999–1.001	50	5	2.5
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	0.999–1.001	50	5	2.5
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0.999–1.001	50	5	2.5
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	0.999–1.001	50	5	2.5
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.986–1.016	50	5	2.5
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0.999–1.001	50	5	2.5
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0.999–1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0.999–1.001	50	5	2.5
OCDF	$^{13}C_{12}$-OCDD	0.995–1.013	100	10	5.0
OCDD	$^{13}C_{12}$-OCDD	0.999–1.001	100	10	5.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.947–0.992			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.940–1.006			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.993–1.017			
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.971–1.000			
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.974–1.002			
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.975–1.006			

Table 2. Retention Times and Minimum Levels for PCDDs and PCDFs (continued)

Compound	Retention Time Reference	Relative Retention Time	Minimum Level[1] Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/µL; ppb)
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.953–1.172			
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.024–1.148			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.023–1.125			
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.050–1.275			

1. The Minimum Level for each analyte is defined as the concentration in a sample equivalent to the concentration of the lowest of the calibration standards analyzed in Section 7, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

Table 3. Descriptors, Exact Masses, m/z Types, and Elemental Compositions of the PCDDs and PCDFs

Descriptor	Accurate m/z[1]	m/z Type	Elemental Composition	Compound[2]
1	292.9825	Lock	$C_7 F_{11}$	PFK
	303.9016	M	$C_{12} H_4 {}^{35}Cl_4 O$	TCDF
	305.8987	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF
	315.9419	M	$^{13}C_{12} H_4 {}^{35}Cl_4 O$	TCDF[3]
	317.9389	M+2	$^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O$	TCDF[3]
	319.8965	M	$C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD
	321.8936	M+2	$C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD
	327.8847	M	$C_{12} H_4 {}^{37}Cl_4 O_2$	TCDD[4]
	330.9792	QC	$C_7 F_{13}$	PFK
	331.9368	M	$^{13}C_{12} H_4 {}^{35}Cl_4 O_2$	TCDD[3]
	333.9339	M+2	$^{13}C_{12} H_4 {}^{35}Cl_3 {}^{37}Cl O_2$	TCDD[3]
	375.8364	M+2	$C_{12} H_4 {}^{35}Cl_5 {}^{37}Cl O$	HxCDPE
2	339.8597	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	341.8567	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF
	351.9000	M+2	$^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O$	PeCDF
	353.8970	M+4	$^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O$	PeCDF[3]
	354.9792	Lock	$C_9 F_{13}$	PFK
	355.8546	M+2	$C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD
	357.8516	M+4	$C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12} H_3 {}^{35}Cl_4 {}^{37}Cl O_2$	PeCDD[3]
	369.8919	M+4	$^{13}C_{12} H_3 {}^{35}Cl_3 {}^{37}Cl_2 O_2$	PeCDD[3]
	409.7974	M+2	$C_{12} H_3 {}^{35}Cl_6 {}^{37}Cl O$	HpCDPE
3	373.8208	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF
	375.8178	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O$	HxCDF
	383.8639	M	$^{13}C_{12} H_2 {}^{35}Cl_6 O$	HxCDF[3]
	385.8610	M+2	$^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O$	HxCDF[3]
	389.8157	M+2	$C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD
	391.8127	M+4	$C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD
	392.9760	Lock	$C_9 F_{15}$	PFK
	401.8559	M+2	$^{13}C_{12} H_2 {}^{35}Cl_5 {}^{37}Cl O_2$	HxCDD[3]
	403.8529	M+4	$^{13}C_{12} H_2 {}^{35}Cl_4 {}^{37}Cl_2 O_2$	HxCDD[3]
	430.9729	QC	$C_9 F_{17}$	PFK
	445.7555	M+4	$C_{12} H_2 {}^{35}Cl_6 {}^{37}Cl_2 O$	OCDPE

Table 3. Descriptors, Exact Masses, m/z Types, and Elemental Compositions of the PCDDs and PCDFs (continued)

Descriptor	*Accurate m/z*[1]	*m/z Type*	*Elemental Composition*	*Compound*[2]
4	407.7818	M+2	C_{12} H $^{35}Cl_6$ ^{37}Cl O	HpCDF
	409.7789	M+4	C_{12} H $^{35}Cl_5$ $^{37}Cl_2$ O	HpCDF
	417.8253	M	$^{13}C_{12}$ H $^{35}Cl_7$ O	HpCDF[3]
	419.8220	M+2	$^{13}C_{12}$ H $^{35}Cl_6$ ^{37}Cl O	HpCDF[3]
	423.7766	M+2	C_{12} H $^{35}Cl_6$ ^{37}Cl O_2	HpCDD
	425.7737	M+4	C_{12} H $^{35}Cl_5$ $^{37}Cl_2$ O_2	HpCDD
	430.9729	Lock	C_9 F_{17}	PFK
	435.8169	M+2	$^{13}C_{12}$ H $^{35}Cl_6$ ^{37}Cl O_2	HpCDD[3]
	437.8140	M+4	$^{13}C_{12}$ H $^{35}Cl_5$ $^{37}Cl_2$ O_2	HpCDD[3]
	479.7165	M+4	C_{12} H $^{35}Cl_7$ $^{37}Cl_2$ O	NCDPE
5	441.7428	M+2	C_{12} $^{35}Cl_7$ ^{37}Cl O	OCDF
	442.9728	Lock	C_{10} F_{17}	PFK
	443.7399	M+4	C_{12} $^{35}Cl_6$ $^{37}Cl_2$ O	OCDF
	457.7377	M+2	C_{12} $^{35}Cl_7$ ^{37}Cl O_2	OCDD
	459.7348	M+4	C_{12} $^{35}Cl_6$ $^{37}Cl_2$ O_2	OCDD
	469.7779	M+2	$^{13}C_{12}$ $^{35}Cl_7$ ^{37}Cl O_2	OCDD[3]
	471.7750	M+4	$^{13}C_{12}$ $^{35}Cl_6$ $^{37}Cl_2$ O_2	OCDD[3]
	513.6775	M+4	C_{12} $^{35}Cl_8$ $^{37}Cl_2$ O	DCDPE

1. Nuclidic masses used:

   H = 1.007825 C = 12.00000 ^{13}C = 13.003355 F = 18.9984
   O = 15.994915 ^{35}Cl = 34.968853 ^{37}Cl = 36.965903

2. | | | | | | |
   |---|---|---|---|---|---|
   | TCDD | = | Tetrachlorodibenzo-*p*-dioxin | TCDF | = | Tetrachlorodibenzofuran |
   | PeCDD | = | Pentachlorodibenzo-*p*-dioxin | PeCDF | = | Pentachlorodibenzofuran |
   | HxCDD | = | Hexachlorodibenzo-*p*-dioxin | HxCDF | = | Hexachlorodibenzofuran |
   | HpCDD | = | Heptachlorodibenzo-*p*-dioxin | HpCDF | = | Heptachlorodibenzofuran |
   | OCDD | = | Octachlorodibenzo-*p*-dioxin | OCDF | = | Octachlorodibenzofuran |
   | HxCDPE | = | Hexachlorodiphenyl ether | HpCDPE | = | Heptachlorodiphenyl ether |
   | OCDPE | = | Octachlorodiphenyl ether | NCDPE | = | Nonachlorodiphenyl ether |
   | DCDPE | = | Decachlorodiphenyl ether | PFK | = | Perfluorokerosene |

3. Labeled compound
4. There is only one m/z for $^{37}Cl_4$-2,3,7,8,-TCDD (cleanup standard).

Table 3A. Theoretical Ion Abundance Ratios and QC Limits

Number of Chlorine Atoms	m/z's Forming Ratio	Theoretical Ratio	QC Limits[1]	
			Lower	Upper
4[2]	M/M+2	0.77	0.65	0.89
5	M+2/M+4	1.55	1.32	1.78
6	M+2/M+4	1.24	1.05	1.43
6[3]	M/M+2	0.51	0.43	0.59
7	M+2/M+4	1.05	0.88	1.20
7[4]	M/M+2	0.44	0.37	0.51
8	M+2/M+4	0.89	0.76	1.02

1. QC limits represent ±15% windows around the theoretical ion abundance ratios.
2. Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).
3. Used for $^{13}C_{12}$-HxCDF only.
4. Used for $^{13}C_{12}$-HpCDF only.

Table 4. Concentration of Solutions Containing Lableled and Unlabeled PCDDs and PCDFs—Stock Standards and Spiking Solutions

Compound	Labeled Compound Stock Solution[1] (ng/mL)	Labeled Compound Spiking Solution[2] (ng/mL)	PAR Stock Solution[3] (ng/mL)	Cleanup Standard Spiking Solution[4] (ng/mL)	Internal Standard Spiking Solution[5] (ng/mL)
2,3,7,8-TCDD	--	--	40	--	--
2,3,7,8-TCDF	--	--	40	--	--
1,2,3,7,8-PeCDD	--	--	200	--	--
1,2,3,7,8-PeCDF	--	--	200	--	--
2,3,4,7,8-PeCDF	--	--	200	--	--
1,2,3,4,7,8-HxCDD	--	--	200	--	--
1,2,3,6,7,8-HxCDD	--	--	200	--	--
1,2,3,7,8,9-HxCDD	--	--	200	--	--
1,2,3,4,7,8-HxCDF	--	--	200	--	--
1,2,3,6,7,8-HxCDF	--	--	200	--	--
1,2,3,7,8,9-HxCDF	--	--	200	--	--
2,3,4,6,7,8-HxCDF	--	--	200	--	--
1,2,3,4,6,7,8-HpCDD	--	--	200	--	--
1,2,3,4,6,7,8-HpCDF	--	--	200	--	--
1,2,3,4,7,8,9-HpCDF	--	--	200	--	--
OCDD	--	--	400	--	--
OCDF	--	--	400	--	--
$^{13}C_{12}$-2,3,7,8-TCDD	100	2	--	--	--
$^{13}C_{12}$-2,3,7,8-TCDF	100	2	--	--	--
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	2	--	--	--
$^{13}C_{12}$-PeCDF	100	2	--	--	--
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	2	--	--	--
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	2	--	--	--
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	2	--	--	--
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	2	--	--	--
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	2	--	--	--
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	2	--	--	--
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	2	--	--	--
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	2	--	--	--
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	2	--	--	--
$^{13}C_{12}$-OCDD	200	4	--	--	--
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	--	--	--	0.2	--
Internal Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	--	--	--	--	200
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	--	--	--	--	200

1. Section 6.10—prepared in nonane and diluted to prepare spiking solution.
2. Section 10.3.2—prepared from stock solution daily.
3. Section 6.14—prepared in nonane and diluted to prepare spiking solution in Section 10.3.4.
4. Section 6.11—prepared in nonane.
5. Section 6.12—prepared in nonane.

Table 4 (continued). Concentration of Solutions Containing Lableled and Unlabeled PCDDs and PCDFs—Calibration and Verification Solutions

Compound	CS1 (ng/mL)	CS2 (ng/mL)	VER[6] CS3 (ng/mL)	CS4 (ng/mL)	CD5 (ng/mL)
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-PeCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	0.5	2	10	40	200
Internal Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

6. Section 14.3—calibration verification solution.

Table 5. GC Retention Time Window Defininig Solutions and Isomer Specificity Test Standards

DB-5 Column GC Retention-Time Window Defining Solution (Section 6.15)

Congener	*First Eluted*	*Last Eluted*
TCDF	1,3,6,8-	1,2,8,9-
TCDD	1,3,6,8-	1,2,8,9-
PeCDF	1,3,4,6,8-	1,2,3,8,9-
PeCDD	1,2,4,7,9-	1,2,3,8,9-
HxCDF	1,2,3,4,6,8-	1,2,3,4,8,9-
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7-
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-

DB-5 Column TCDD Specificity Test Standard (Section 6.16.1)

1,2,3,4-TCDD
1,2,7,8-TCDD
1,4,7,8-TCDD
1,2,3,7-TCDD
1,2,3,8-TCDD
2,3,7,8-TCDD

DB-225 Column TCDF Isomer Specificity Test Standard (Section 6.16.2)

2,3,4,7-TCDF
2,3,7,8-TCDF
1,2,3,9-TCDF

Table 6. Reference Compounds for Quantitation of Unlabeled and Labeled PCDDs and PCDFs

Compound	*Reference for Quantitation*
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD	--[1]
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF
OCDD	$^{13}C_{12}$-OCDD
OCDF	$^{13}C_{12}$-OCDD
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD

1. 1,2,3,7,8,9-HxCDD is quantified using the average responses for the $^{13}C_{12}$-1,2,3,4,7,8-HxCDD and the $^{13}C_{12}$-1,2,3,6,7,8-HxCDD.

Table 7. Acceptance Criteria for Performance Tests

Compound	Test Conc.[1] (ng/mL)	IPR[2] s (ng/mL)	IPR[2] X (ng/mL)	OPR[2] (ng/mL)	VER (ng/mL)
2,3,7,8-TCDD	10	1.1	8.0–12.5	6.7–14.4	8.4–11.9
2,3,7,8-TCDF	10	0.5	8.2–12.8	6.6–17.3	9.2–10.9
1,2,3,7,8-PeCDD	50	1.5	44.2–53.1	38.6–65.7	41.3–60.5
1,2,3,7,8-PeCDF	50	1.5	44.1–55.2	38.4–66.6	41.2–60.6
2,3,4,7,8-PeCDF	50	3.4	45.7–58.7	36.5–70.0	42.9–58.3
1,2,3,4,7,8-HxCDD	50	5.3	40.6–64.6	34.8–79.5	45.8–54.6
1,2,3,6,7,8-HxCDD	50	3.7	47.5–50.6	49.5–63.2	45.8–54.6
1,2,3,7,8,9-HxCDD	50	5.6	35.6–73.9	15.7–168.0	38.7–64.6
1,2,3,4,7,8-HxCDF	50	3.7	41.7–54.5	39.3–56.4	41.4–60.4
1,2,3,6,7,8-HxCDF	50	1.9	47.0–54.2	38.8–65.5	43.0–58.2
1,2,3,7,8,9-HxCDF	50	3.6	46.6–54.0	36.8–59.1	44.3–56.5
2,3,4,6,7,8-HxCDF	50	2.2	44.8–52.8	37.4–62.8	45.0–55.6
1,2,3,4,6,7,8-HpCDD	50	3.3	39.6–58.0	38.3–65.0	44.3–56.4
1,2,3,4,6,7,8-HpCDF	50	2.6	43.9–55.4	30.4–84.2	48.2–51.9
1,2,3,4,7,8,9-HpCDF	50	2.9	49.5–52.1	32.2–80.1	46.5–53.8
OCDD	100	11.3	73.8–149.1	76.2–156.2	78.8–126.9
OCDF	100	5.8	74.0–128.7	54.7–285.9	76.7–130.4
$^{13}C_{12}$-2,3,7,8-TCDD	100	16.0	25.0–150.0	25.0–150.0	86.3–124.5
$^{13}C_{12}$-2,3,7,8-TCDF	100	18.4	25.0–150.0	25.0–150.0	77.5–129.0
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	21.2	25.0–150.0	25.0–150.0	71.9–139.1
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	15.9	25.0–150.0	25.0–150.0	81.3–123.0
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	20.1	25.0–150.0	25.0–150.0	82.2–121.6
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	18.7	25.0–150.0	25.0–150.0	90.8–110.1
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	100	24.1	25.0–150.0	25.0–150.0	85.6–116.9
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	14.5	25.0–150.0	25.0–150.0	83.1–120.4
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	11.5	25.0–150.0	25.0–150.0	73.1–136.7
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	14.8	25.0–150.0	25.0–150.0	80.0–124.9
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	10.4	25.0–150.0	25.0–150.0	80.0–124.9
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	20.4	25.0–150.0	25.0–150.0	69.5–143.8
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	18.8	25.0–150.0	25.0–150.0	89.3–112.0
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	22.9	25.0–150.0	25.0–150.0	77.4–129.3
$^{13}C_{12}$-OCDD	200	43.9	50.0–300.0	50.0–300.0	105.2-380.1
$^{37}Cl_4$-2,3,7,8-TCDD	10	--	2.5–15.0	2.5–15.0	7.8–12.8

1. All specifications are given as concentration in the final extract, assuming a 20 µL volume.
2. s = standard deviation of the concentration, X = average concentration. Concentration limits for labeled compounds in the IPR and OPR aliquots are based on requirements for labeled compound recovery of 25 to 150% (Sections 8.2.3 and 14.5.3).

Table 8. Sample Phase and Quantity Extracted for Various Matrices

Sample Matrix[1]	*Example*	*Percent Solids*	*Phase*	*Quantity Extracted*
Single-phase				
Aqueous	Drinking Water Groundwater Treated Wastewater	<1	--[2]	1000 mL
Solid	Dry Soil Compost Ash	>20	Solid	10 g
Organic	Waste Solvent Waste Oil Organic Polymer	<1	Organic	10 g
Multi-phase				
Liquid/Solid				
Aqueous/Solid	Wet Soil Untreated effluent Digested municipal sludge Filter cake Paper pulp Tissue	1–30	Solid	10 g
Organic/solid	Industrial sludge Oily waste	1–100	Both	10 g
Liquid/Liquid				
Aqueous/organic	In-process effluent Untreated effluent Drum waste	<1	Organic	10 g
Aqueous/organic/solid	Untreated effluent Drum waste	>1	Organic & solid	10 g

1. The extract matrix may be vague for some samples. In general, when the CDDs and CDFs are in contact with a multiphase system in which one of the phases is water, they will be preferentially dispersed in or adsorbed on the alternate phase, because of their low solubility in water.
2. Aqueous samples are filtered after spiking with labeled analogs. The filtrate and the materials trapped on the filter are extracted separately, and then the extracts are combined for cleanup and analysis.

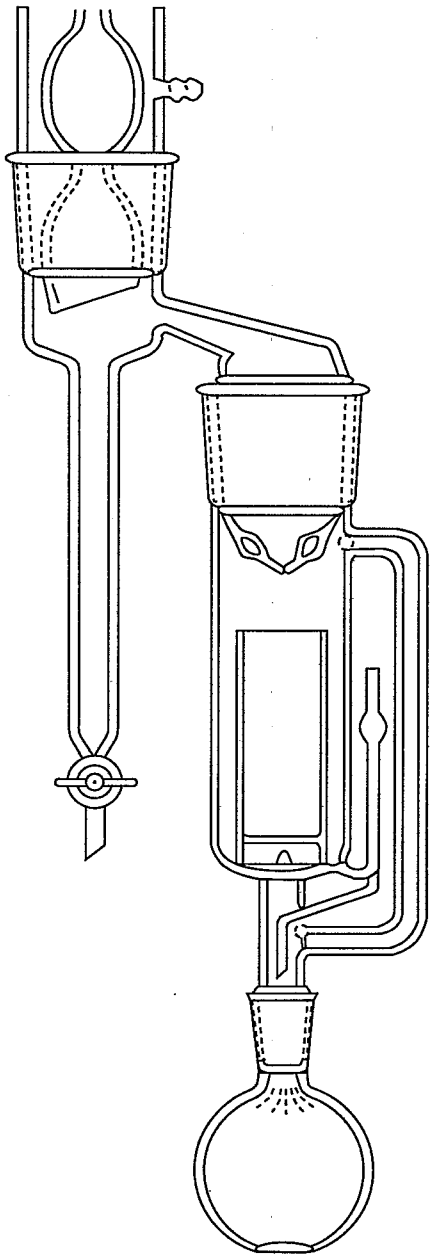

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Figure 1. Soxhlet/Dean-Stark Extractor

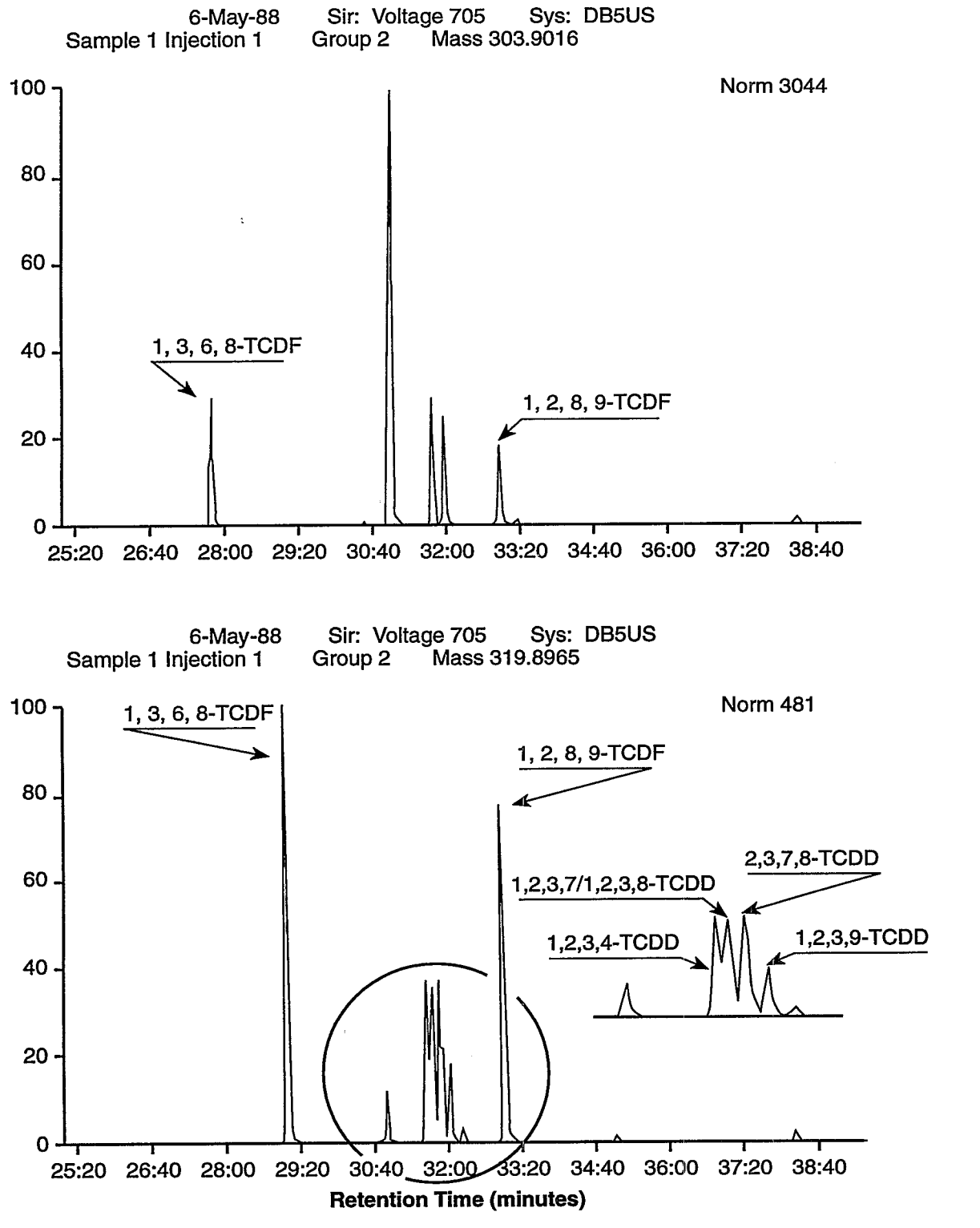

Figure 2A. First- and Last-Eluted Tetra-Dioxin and -Furan Isomers

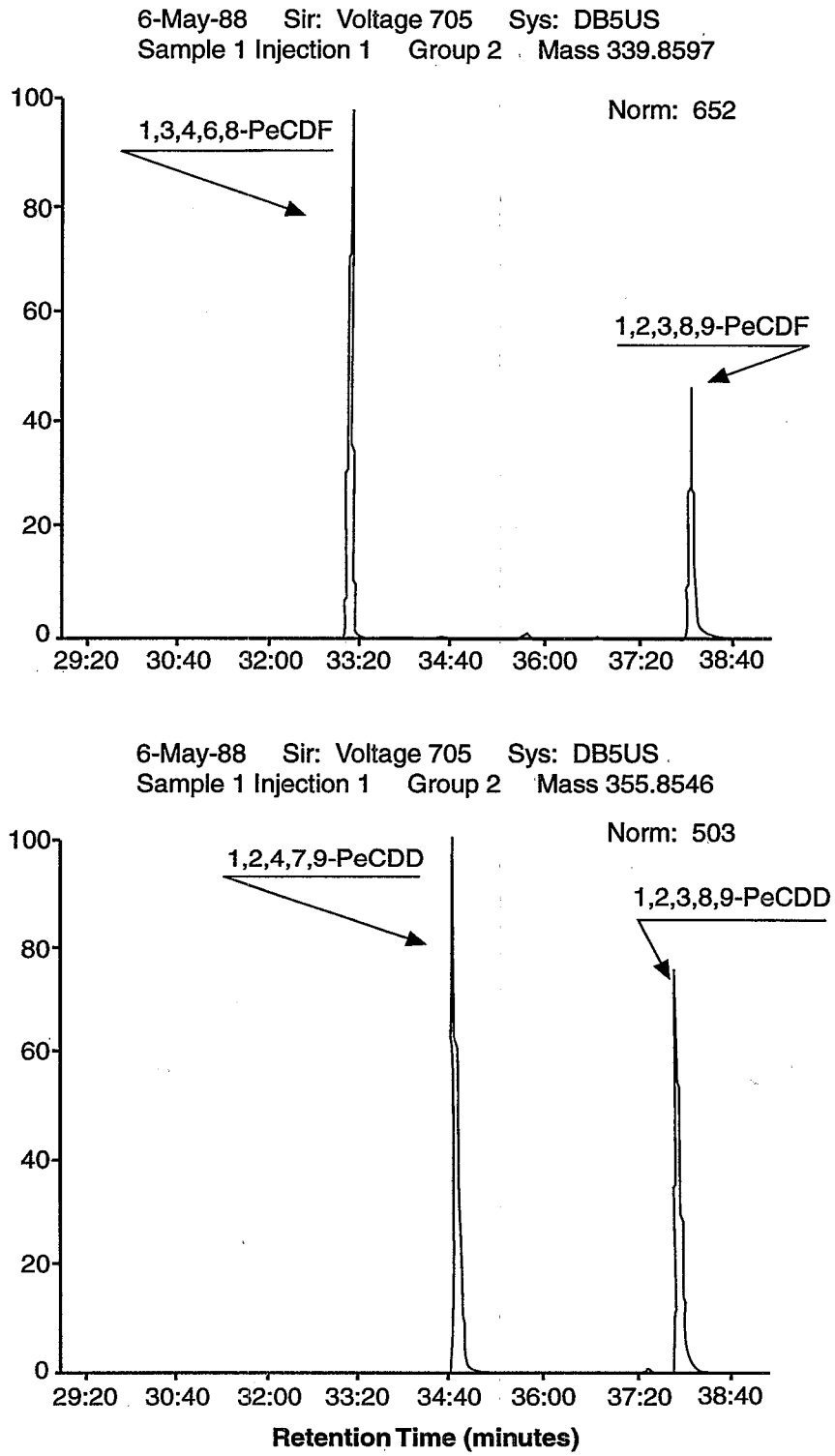

Figure 2B. First- and Last-Eluted Penta-Dioxin and -Furan Isomers

52-020-04A

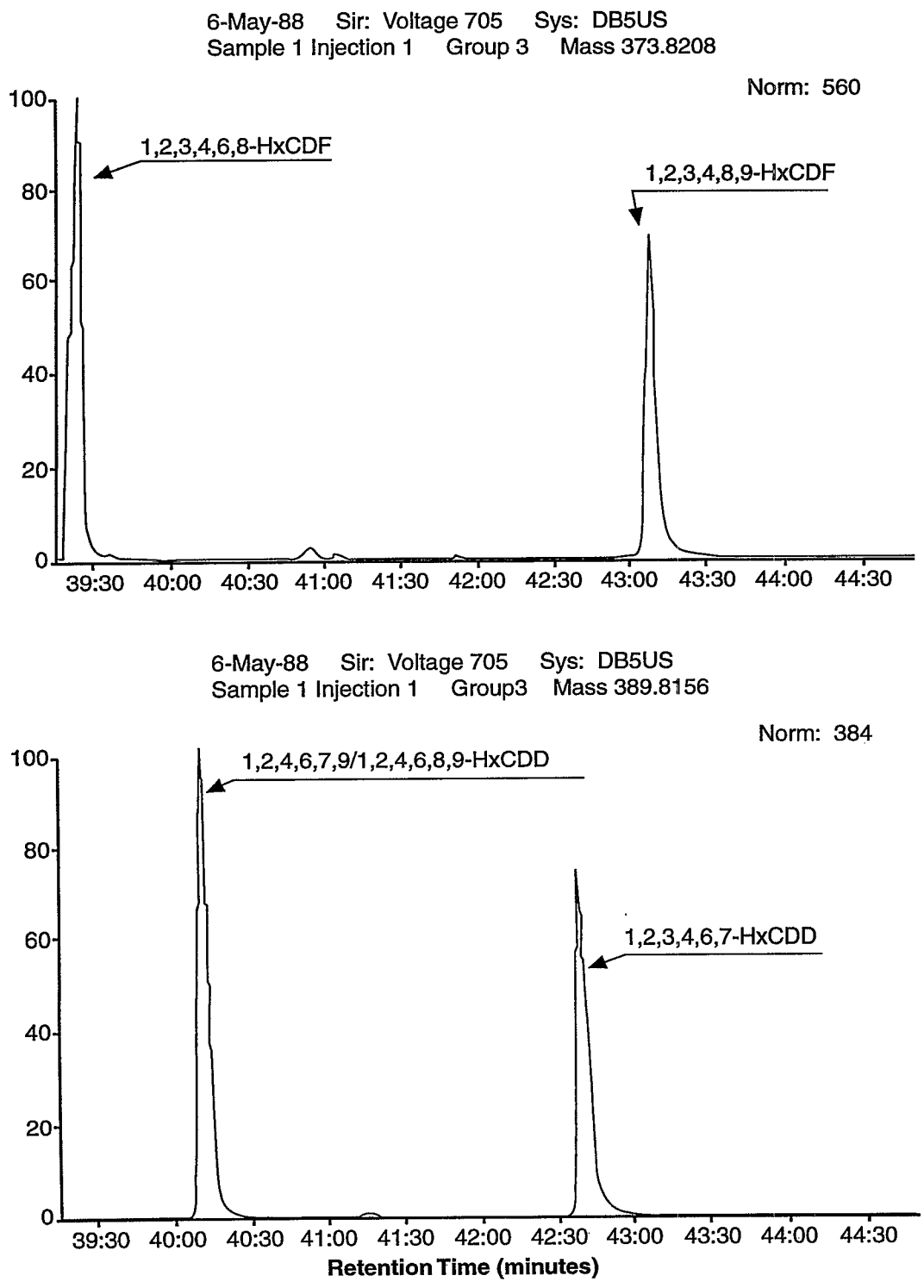

Figure 2C. First- and Last-Eluted Hexa-Dioxin and -Furan Isomers

52-020-05A

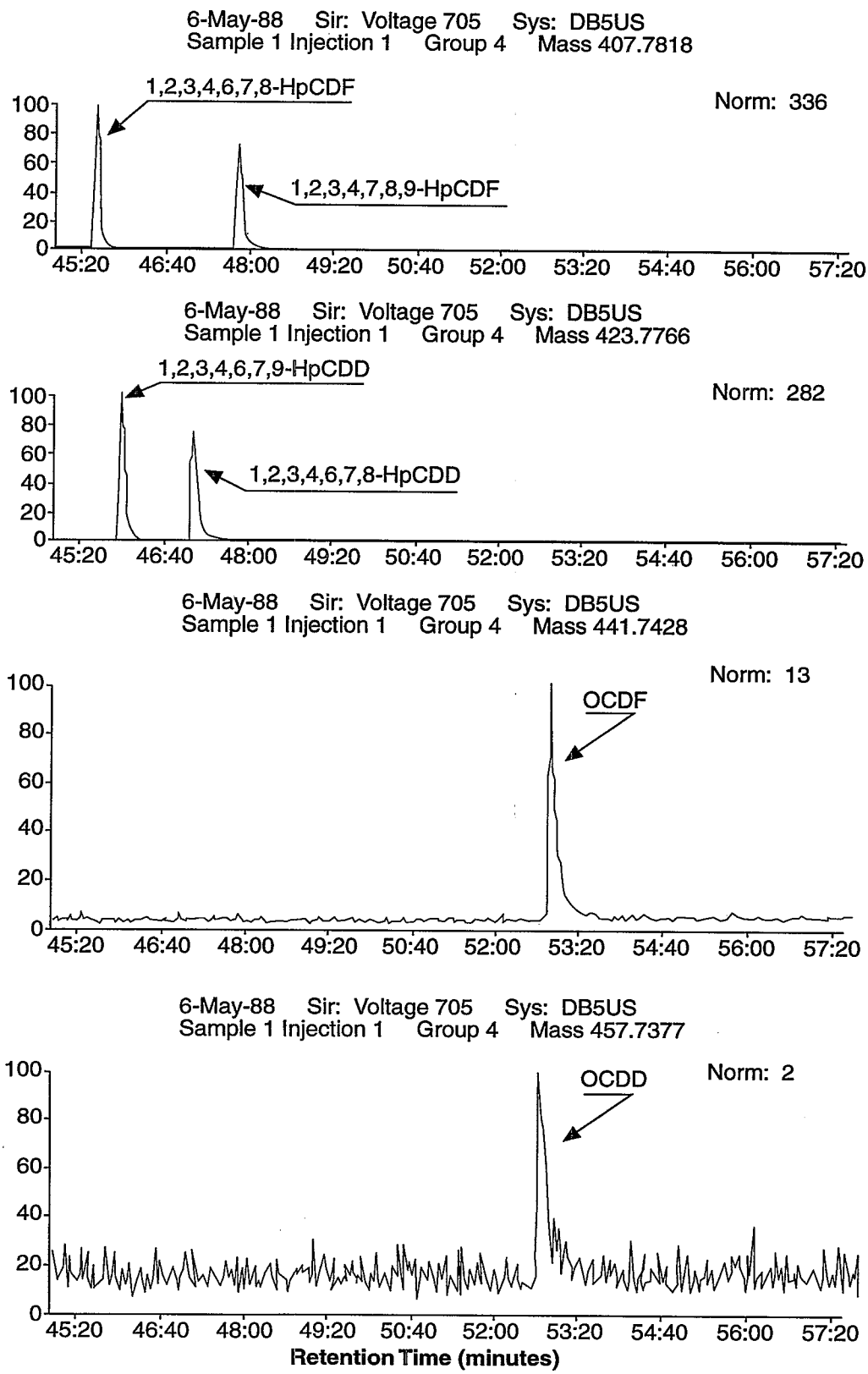

Figure 2D. First- and Last-Eluted Hepta-Dioxin and -Furan Isomers

52-020-06A

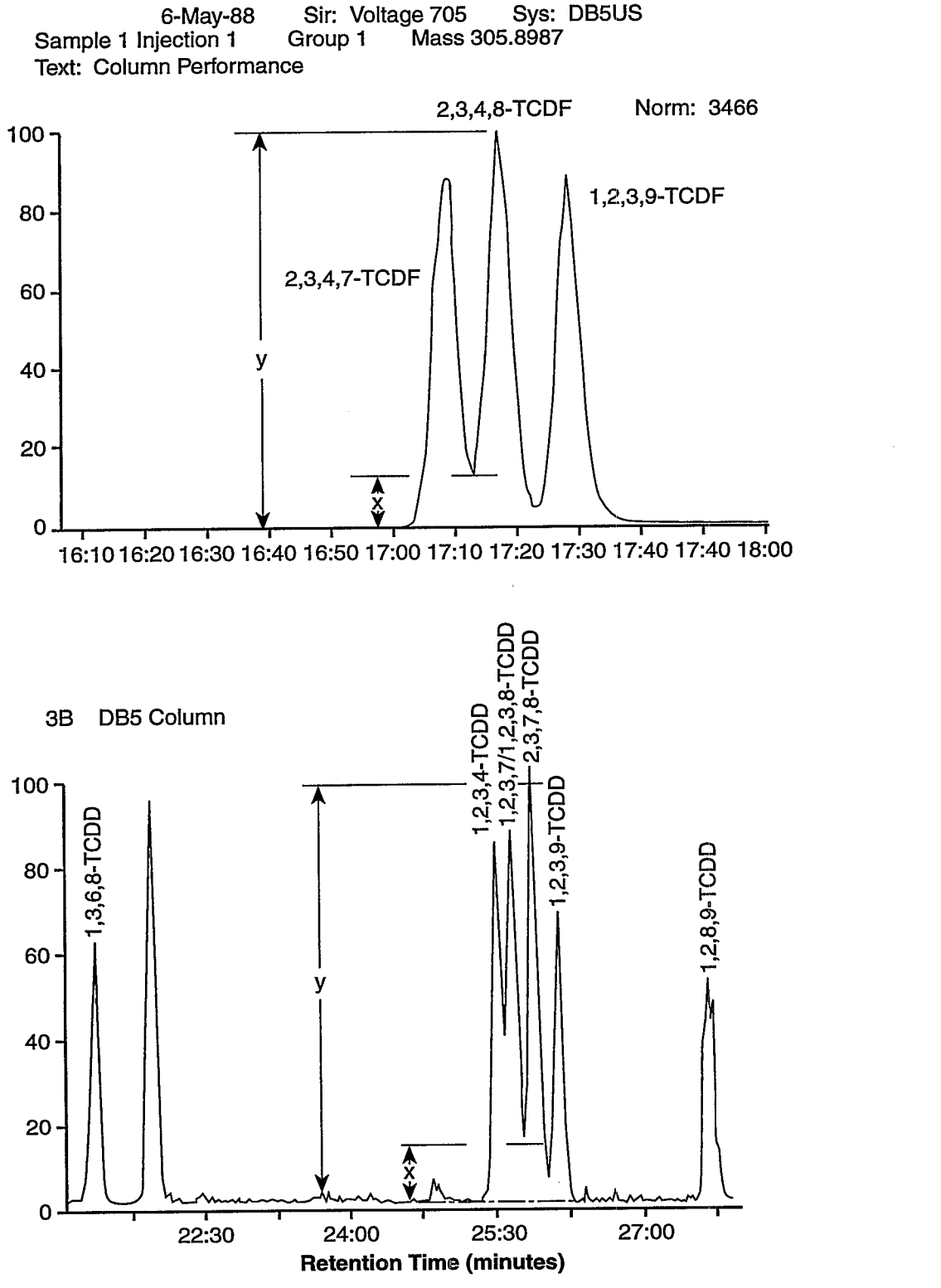

Figure 3. Valley Between 2,3,7,8- Tetra-Dioxin and -Furan Isomers and Other Closely Eluted Isomers

Appendix to Method 1613

Modifications to Method 1613 for the Analysis of 2,3,7,8-TCDD and 2,3,7,8-TCDF Only

This appendix lists the modifications to Method 1613 for analysis of the 2,3,7,8 isomers of TCDD and TCDF without analysis of the other fifteen 2,3,7,8-substituted dioxin and furan isomers. Modifications are given on a paragraph-by-paragraph basis. Any modifications made by the laboratory must be validated by performing the startup tests described in Section 8 of the existing method, and by meeting all of the relevant performance specifications in Table 7.

The modifications outlined below limit performance measures in the method to those that involve 2,3,7,8-TCDD and 2,3,7,8-TCDF (referred to below as TCDD and TCDF, respectively). An "a" suffix has been added to the section numbers to identify the sections that have been modified.

6.7a The standard solutions are modified to include unlabeled and labeled TCDD and TCDF, and $^{37}Cl_4$-2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD.

6.8a The stock solutions in this section are modified to be consistent with the solutions in Section 6.7a.

6.9a The secondary standard solutions are modified to be consistent with the solutions in Section 6.7a.

6.10a The labeled-compound stock standard is modified to contain $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-2,3,7,8-TCDF only.

6.13a The calibration solutions are modified to contain unlabeled and labeled TCDD and TCDF only.

6.14a The PAR solution is modified to contain TCDD and TCDF only.

6.15a The window-defining mixture is modified to include TCDD and TCDF only.

7.1.1a The temperature program may be modified to optimize the GC run for separation of the tetrachloro-dioxins and -furans, and to shorten the run as appropriate.

7.2a The minimum level, ion-abundance ratio, signal-to-noise ratio, and retention-time specifications evaluated here apply to TCDD and TCDF (unlabeled and labeled) only.

7.2.1a The ion-abundance ratios apply to TCDD and TCDF (unlabeled and labeled) only.

7.2.1.1a The ion descriptors may be modified to include only the tetra- and penta-isomers, the diphenyl ether ions, and the lock-mass ions.

7.2.3a The signal-to-noise specifications and the minimum level specifications apply to TCDD and TCDF only.

7.3a The retention-time window standard need contain only the tetra-isomers specified in the table.

7.5a The calibration requirements apply to TCDD and TCDF only.

8.2a The IPR requirements apply to TCDD and TCDF only and must be performed using the same procedures used to generate succeeding sample data, i.e., any modifications made to the procedures must be verified by a new IPR test.

8.5.2a The acceptance criteria for blanks apply to TCDD and TCDF only and to any interferences with the analysis of those compounds.

8.7a If spiked sample analysis is requested, spike only TCDD and TCDF.

12.3a Silica column may be optimized for TCDD and TCDF.

12.4a Alumina column cleanup may be optimized for TCDD and TCDF.

12.5a AX-21 column cleanup may be optimized for TCDD and TCDF.

13.3a The GC temperature program and data collection parameters may be optimized for TCDD and TCDF.

14.3a Calibration verification applies to unlabeled and labeled TCDD and TCDF only.

14.4.1.1a Criteria for $^{13}C_{12}$-1,2,3,7,8,9-HxCDD do not apply.

14.5a The OPR solution contains TCDD and TCDF only, and only those compounds are evaluated.

15A Confirmatory-column analysis is required when 2,3,7,8-TCDF is detected.

16.1a 2,3,7,8-TCDD and 2,3,7,8-TCDF are determined by isotope dilution.

16.1.1a Does not apply.

16.1.2a Does not apply.

Method 1650

Adsorbable Organic Halides by Adsorption and Coulometric Titration

Revision B, October 1993

Method 1650

Adsorbable Organic Halides by Adsorption and Coulometric Titration

1. SCOPE AND APPLICATION

1.1 This method is designed to meet the survey requirements of the United States Environmental Protection Agency (EPA). It is used to determine organic halides associated with the Clean Water Act; the Resource Conservation and Recovery Act; the Comprehensive Environmental Response, Compensation, and Liability Act; and other organic halides amenable to combustion and coulometric titration.

1.2 The method is applicable to the determination of organic halides in water and wastewater. However, the method is applicable to the determination of in-process Pulp and Paper waste streams only when using the column technique stated herein. This method is a combination of several existing methods for organic halide measurements (References 1 through 7).

1.3 The method can be used to measure organically-bound halides (chlorine, bromine, iodine) present in dissolved or suspended form. Results are reported as organic chloride (Cl^-). The detection limit of the method is usually dependent on interferences rather than instrumental limitations. A method detection limit (MDL; Reference 8) of 6.6 μg/L, and a minimum level (ML; the level at which the entire analytical system must give reliable signals and an acceptable calibration point) of 20 μg/L, can be achieved with no interferences present.

1.4 This method is for use by or under the supervision of analysts experienced in the use of a combustion/micro-coulometer. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedures described in Section 8.2.

1.5 Any modification of the method beyond those expressly permitted (Section 8.1.2) is subject to application and approval of an alternate test procedure under 40 *CFR* Parts 134 and 135.

2. SUMMARY OF METHOD

2.1 Sample preservation: Residual chlorine that may be present is removed by the addition of sodium thiosulfate. Samples are adjusted to a pH <2 and maintained at 0 to 4°C until analysis.

2.2 Sample analysis: The organic halide in water is determined by adsorption onto granular activated carbon (GAC), washing the adsorbed sample and GAC to remove inorganic halide, combustion of the sample and GAC to form the hydrogen halide, and titration of the hydrogen halide with a micro-coulometer, as shown in Figure 4.

2.3 Micro-coulometer.

2.3.1 This detector operates by maintaining a constant silver-ion concentration in a titration cell. An electric potential is applied to a solid silver electrode to produce silver ions in the cell. As hydrogen halide produced from the combustion of organic halide enters the cell, it is partitioned into an acetic acid electrolyte where it precipitates as silver halide. The current produced is integrated over the combustion period. The

electric charge is proportional to the number of moles of halogen captured in the cell (Reference 6).

2.3.2 The mass concentration of organic halides is reported as an equivalent concentration of organically bound chloride (Cl^-).

3. CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield elevated readings from the micro-coulometer. All materials 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 set (samples started through the adsorption process in a given 8 hour shift, to a maximum of 20 samples). Specific selection of reagents and purification of solvents may be required.

3.2 Glassware is cleaned by detergent washing in hot water, rinsing with tap water and distilled water, capping with aluminum foil, and baking at 450°C for at least 1 hour. For some glassware, immersion in a chromate cleaning solution prior to detergent washing may be required. If blanks from glassware without cleaning or with fewer cleaning steps show no detectable organic halide, the cleaning steps from above that do not eliminate organic halide may be omitted.

3.3 Most often, contamination results from methylene chloride vapors in laboratories that perform organic extractions. Heating, ventilating, and air conditioning systems that are shared between the extraction laboratory and the laboratory in which organic halide measurements are performed transfer the methylene chloride vapors to the air in the organic halide laboratory. Exposure of the activated carbon used in the analysis results in contamination. Separate air handling systems, charcoal filters, and glove boxes can be used to minimize this exposure.

3.4 Activated carbon.

3.4.1 The purity of each lot of activated carbon must be verified before each use by measuring the adsorption capacity and the background level of halogen (Section 8.5). The stock of activated carbon should be stored in its granular form in a glass container that is capped tightly. Protect carbon at all times from sources of halogen vapors.

3.4.2 Inorganic substances such as chloride, chlorite, bromide, and iodide will adsorb on activated carbon to an extent dependent on their original concentration in the aqueous solution and the volume of sample adsorbed. Treating the activated carbon with a solution of nitrate causes competitive desorption of inorganic halide species. However, if the inorganic halide concentration is greater than 2,000 times the organic halide concentration, artificially high results may be obtained.

3.4.3 Halogenated organic compounds that are weakly adsorbed on activated carbon are only partially recovered from the sample. These include certain alcohols and acids such as chloroethanol and chloroacetic acid that can be removed from activated carbon by the nitrate wash.

3.5 Polyethylene gloves should be worn when handling equipment surfaces in contact with the sample.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical substance should be treated as a potential health hazard. Exposure to these substances should be reduced to the lowest possible level. 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 safety data sheets should be made available to all personnel involved in the chemical analysis. Additional information on laboratory safety can be found in References 9 through 11.

4.2 This method employs strong acids. Appropriate clothing, gloves, and eye protection should be worn when handling these substances.

4.3 Field samples may contain high concentrations of toxic volatile compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment.

5.1.1 Bottle: 500-mL minimum, amber glass. Detergent water wash, chromic acid rinse, rinse with tap and distilled water, cover with aluminum foil and heat to 450°C for at least 1 hour before use.

5.1.2 PTFE liner: Cleaned as above and baked at 100 to 200°C for at least 1 hour.

5.1.3 Bottles and liners must be lot certified to be free of organic halide by running blanks according to this method.

5.2 Scoop for granular activated carbon (GAC): Capable of precisely measuring 0.13 cc (±0.01 - cc) GAC (Dohrmann Measuring Cup 521-021, or equivalent). This scoop size has been shown to hold 35 to 60 mg of GAC, depending on the carbon source. The variance in GAC mass has been shown to have no affect on method performance (Reference 13).

5.3 Batch adsorption and filtration system.

5.3.1 Adsorption system: Rotary shaker, wrist action shaker, ultrasonic system, or other system for assuring thorough contact of sample with activated carbon. Systems different from the one described below must be demonstrated to meet the performance requirements in Section 8 of this method.

5.3.1.1 Erlenmeyer flasks: 250- to 500-mL with ground-glass stopper, for use with rotary shaker.

5.3.1.2 Shake table: Sybron Thermolyne Model LE "Big Bill" rotator/shaker, or equivalent.

5.3.1.3 Rack attached to shake table to permit agitation of 16 to 25 samples simultaneously.

5.3.2 Filtration system (Figure 1).

5.3.2.1 Vacuum filter holder: Glass, with fritted-glass support (Fisher Model 09-753E, or equivalent).

5.3.2.2 Polycarbonate filter: 0.45 micron, 25 mm diameter (Micro Separations Inc, Model K04CP02500, or equivalent).

5.3.2.3 Filter forceps: Fisher Model 09-753-50, or equivalent, for handling filters. Two forceps may better aid in handling filters. Clean by washing with detergent and water, rinsing with tap and deionized water, and air drying on aluminum foil.

5.3.2.4 Vacuum flask: 500-mL (Fisher 10-1800, or equivalent).

5.3.2.5 Vacuum Source: A pressure/vacuum pump, rotary vacuum pump, or other vacuum source capable of providing at least 610 mm (24 in) Hg vacuum and 30 L/min free air displacement.

5.3.2.6 Stopper and tubing to mate the filter holder to the flask and the flask to the pump.

5.3.2.7 Polyethylene gloves: (Fisher 11-394-110-B, or equivalent).

5.4 Column adsorption system.

5.4.1 Adsorption module: Dohrmann AD-2, Mitsubishi TXA-2, or equivalent with pressurized sample and nitrate-wash reservoirs, adsorption columns, column housings, gas and gas pressure regulators, and receiving vessels. For each sample reservoir, there are two adsorption columns connected in series. A small steel funnel for filling the columns and a rod for pushing out the carbon are also required. A schematic of the column adsorption system is shown in Figure 2.

5.4.2 Adsorption columns: Pyrex, 4 to 5 cm long × 2 mm I.D. to hold 40 mg of granular activated carbon (GAC).

5.4.3 Cerafelt: Johns-Manville, or equivalent, formed into plugs using stainless steel borer (2 mm I.D.) with ejection rod (available from Dohrmann or Mitsubishi) to hold 40 mg of granular activated carbon (GAC). Caution: Handle Cerafelt with gloves.

5.4.4 Column holders: To support adsorption columns.

5.5 Combustion/micro-coulometer system: Commercially available as a single unit or assembled from parts. At the time of writing of this method, organic halide units were commercially available from Dohrmann Division of Rosemount Analytical, Santa Clara, California; Euroglas BV, Delft, the Netherlands; and Mitsubishi Chemical Industries Ltd., Tokyo, Japan.

5.5.1 Combustion system: Older systems may not have all of the features shown in Figure 4. These older systems may be used provided the performance requirements (Section 8) of this method are met.

5.5.1.1 Combustion tube: Quartz, capable of being heated to 800 to 1000°C and accommodating a boat sampler. The tube must contain an air lock for introduction of a combustion boat, connections for purge and combustion gas, and connection to the micro-coulometer cell.

5.5.1.2 Tube furnace capable of controlling combustion tube in the range of 800 to 1000°C.

5.5.1.3 Boat sampler: Capable of holding 35 to 60 mg of activated carbon and a polycarbonate filter, and fitting into the tube (5.5.1.1). Some

manufacturers offer an enlarged boat and combustion tube for this purpose. Under a time-controlled sequence, the boat is first moved into an evaporation zone where water and other volatiles are evaporated, and then into the combustion zone where the carbon and all organic material in the boat is burned in a flowing oxygen stream. The evolved gases are transported by a non-reactive carrier gas to the micro-coulometer cell.

5.5.1.4 Motor driven boat sampler: Capable of advancing the combustion boat into the furnace in a reproducible time sequence. A suggested time sequence is as follows:

A. Establish initial gas flow rates: 160 mL/min CO_2; 40 mL/min O_2.
B. Sequence start.
C. Hold boat in hatch for 5 seconds to allow integration for baseline subtraction.
D. Advance boat into vaporization zone.
E. Hold for boat in vaporization zone for 110 seconds.
F. Establish gas flow rates for combustion: 200 mL/min O_2; 0 mL/min CO_2; advance boat into pyrolysis zone (800°C).
G. Hold boat in pyrolysis zone for 6 minutes.
H. Return gas flow rates to initial values; retract boat into hatch to cool and to allow remaining HX to be swept into detector (approximately 2 minutes).
I. Stop integration at 10 minutes after sequence start.

NOTE: If the signal from the detector does not return to baseline, it may be necessary to extend the pyrolysis time.

The sequence above may need to be optimized for each instrument.

5.5.1.5 Absorber: Containing sulfuric acid to dry the gas stream after combustion to prevent backflush of electrolyte is highly recommended.

5.5.2 Micro-coulometer system: Capable of detecting the equivalent of 0.2 μg of Cl^- at a signal-to-noise ratio of 2; capable of detecting the equivalent of 1 μg of Cl^- with a relative standard deviation of less than 10%, and capable of accumulating a minimum of the equivalent of 500 μg of Cl^- before a change of electrolyte is required.

5.5.2.1 Micro-coulometer cell: The three cell designs presently in use are shown in Figure 3. Cell operation is described in Section 2.

5.5.2.2 Cell controller: Electronics capable of measuring the small currents generated in the cell and accumulating and displaying the charge produced by hydrogen halides entering the cell. A strip-chart recorder is desirable for display of accumulated charge.

5.6 Miscellaneous glassware.

5.6.1 Volumetric flasks: 5-, 10-, 25-, 50-, 100-, and 1000-mL.

5.6.2 Beakers: 100-, 500-, and 1000-mL.

5.6.3 Volumetric pipets: 1 and 10-mL with pipet bulbs.

5.6.4 Volumetric micro-pipets: 10-, 20-, 50-, 100-, 200-, and 500-μL with pipet control (Hamilton 0010, or equivalent).

5.6.5 Graduated cylinders: 10-, 100-, and 1000-mL.

5.7 Micro-syringes: 10-, 50-, and 100-μL.

5.8 Balances.

5.8.1 Top-loading, capable of weighing 0.1 g.

5.8.2 Analytical, capable of weighing 0.1 mg.

5.9 pH meter.

5.10 Wash bottles: 500- to 1000-mL, PTFE or polyethylene.

6. *Reagents and Standards*

6.1 Granular activated carbon (GAC): 75 to 150 μm (100 to 200 mesh); (Dohrmann, Mitsubishi, carbon plus, or equivalent), with chlorine content less than 1 μg Cl^- per scoop (<25 μg Cl^- per gram), adsorption capacity greater than 1000 μg Cl^- (as 2,4,6-trichlorophenol) per scoop (>25,000 μg/g), inorganic halide retention of less than 1 μg Cl^- per scoop in the presence of 10 mg of inorganic halide (<20 μg Cl^- per gram in the presence of 2500 mg of inorganic halide), and that meets the other test criteria in Section 8.5 of this method.

6.2 Reagent water: Water in which organic halide is not detected by this method.

6.2.1 Preparation: Reagent water may be generated by:

6.2.1.1 Activated carbon: Pass tap water through a carbon bed (Calgon Filtrasorb-300, or equivalent).

6.2.1.2 Water purifier: Pass tap water through a purifier (Millipore Super Q, or equivalent).

6.2.2 pH adjustment: Adjust the pH of the reagent water to <2 with nitric acid for all reagent water used in this method, except for the acetic acid solution (Section 6.13).

6.3 Nitric acid (HNO_3): Concentrated, analytical grade.

6.4 Sodium chloride (NaCl) solution (100 μg/mL of Cl^-): Dissolve 0.165 g NaCl in 1000 mL reagent water. This solution is used for cell testing and for the inorganic halide rejection test.

6.5 Ammonium chloride (NH_4Cl) solution (100 μg/mL of Cl^-): Dissolve 0.1509 g NH_4Cl in 1000 mL reagent water.

6.6 Sulfuric acid: Reagent grade (specific gravity 1.84).

6.7 Oxygen: 99.9% purity.

6.8 Carbon Dioxide: 99.9% purity.

6.9 Nitrate stock solution: In a 1000-mL volumetric flask, dissolve 17 g of $NaNO_3$ in approximately 100 mL of reagent water, add 1.4 mL nitric acid (Section 6.3) and dilute to the mark with reagent water.

6.10 Nitrate wash solution: Dilute 50 mL of nitrate stock solution (Section 6.9) to 1000 mL with reagent water.

6.11 Sodium thiosulfate ($Na_2S_2O_3$) solution (1 N): Weigh 79 grams of $Na_2S_2O_3$ in a 1-L volumetric flask and dilute to the mark with reagent water.

6.12 Trichlorophenol solutions.

6.12.1 Methanol: HPLC grade.

6.12.2 Trichlorophenol stock solution (1.0 mg/mL of Cl^-): Dissolve 0.186 g of 2,4,6-trichlorophenol in 100 mL of halide-free methanol.

6.12.3 Trichlorophenol calibration solutions.

6.12.3.1 Place approximately 900 mL of reagent water in each of five 1000-mL volumetric flasks.

6.12.3.2 Using a calibrated micro-syringe or micro-pipets, add 20, 50, 100, 300, and 800 μL of the trichlorophenol stock solution (Section 6.12.2) to the volumetric flasks and dilute each to the mark with reagent water to produce calibration solutions of 20, 50, 100, 300, and 800 μg Cl^- per liter.

6.12.3.3 Some instruments may have a calibration range that does not extend to 800 μg/L (80 μg of Cl^-). For those instruments, a narrower dynamic range may be used. However, if the concentration of halide in a sample exceeds that range, the sample must be diluted to bring the concentration within the range calibrated.

6.12.4 Trichlorophenol precision and recovery (PAR) test solution (100 μg/L of Cl^-): Place 100 μL of the stock solution (Section 6.12.2) in a 1000-mL volumetric flask and dilute to the mark with reagent water.

6.13 Acetic acid solution: Containing 30 to 70% acetic acid in deionized water per the instrument manufacturer's instructions.

7. CALIBRATION

7.1 Assemble the organic halide (OX) system and establish the operating conditions necessary for analysis. Differences between various makes and models of instruments will require differing operating procedures. Analysts should follow the operating instructions provided by the manufacturer of their particular instrument. Sensitivity, instrument detection limit, precision, linear range, and interference effects must be investigated and established for each particular instrument. Calibration is performed when the instrument is first set up and when calibration cannot be verified (Section 11).

7.2 Cell performance test: Inject 100 μL of the sodium chloride solution (10 μg Cl^-; Section 6.4) directly into the titration cell electrolyte. Adjust the instrument to produce a reading of 10 μg Cl^-.

7.3 Combustion system test: This test can be used to assure that the combustion/micro-coulometer systems are performing properly without introduction of carbon. This test should be used during initial instrument setup and when instrument performance indicates a problem with the combustion system.

7.3.1 Designate a quartz boat for use with the ammonium chloride (NH_4Cl) solution only.

7.3.2 Inject 100 μL of the NH_4Cl solution (Section 6.5) into this boat and proceed with the analysis.

7.3.3 The result shall be between 9.5 and 10.5 μg Cl^-. If the recovery is not between these limits, the combustion or micro-coulometer systems are not performing properly. Check the temperature of the combustion system, and verify that there are no leaks in the combustion system and verify that the cell is performing properly (Section 7.2), then repeat the test.

7.4 Trichlorophenol combustion test: This test can be used to assure that the combustion/micro-coulometer systems are performing properly when carbon is introduced. It should be used during instrument setup and when it is necessary to isolate the adsorption and combustion steps.

7.4.1 Inject 10 μL of the 1 mg/mL trichlorophenol calibration solution (Section 6.12.2) onto one level scoop of GAC in a quartz boat.

7.4.2 Immediately proceed with the analysis to prevent loss of trichlorophenol and to prevent contamination of the carbon.

7.4.3 The result shall be between 9.0 and 11.0 μg Cl^-. If the recovery is not between these limits, the combustion/micro-coulometer system shall be adjusted and the test repeated until the result falls within these limits.

7.5 Background level of Cl^-: Determine the average background level of Cl^- for the entire analytical system as follows:

7.5.1 Using the procedure in Section 10 (batch or column) that will be used for the analysis of samples, determine the background level of Cl^- in each of three 100-mL portions of reagent water.

7.5.2 Calculate the average (mean) concentration of Cl^- and the standard deviation of the concentration.

7.5.3 The sum of the average concentration plus two times the standard deviation of the concentration shall be less than 20 μg/L. If not, the water or carbon shall be replaced, or the adsorption system moved to an area free of organic halide vapors, and the test (Section 7.5) shall be repeated. Only after this test is passed may calibration proceed.

7.6 Calibration by external standard: A calibration curve encompassing the calibration range is performed using 2,4,6-trichlorophenol.

7.6.1 Analyze each of the five calibration solutions (Section 6.12.3) using the procedure in Section 10 (batch or column) that will be used for the analysis of samples, and the same procedure that was used for determination of the system background (Section 7.5). Analyze these solutions beginning with the lowest concentration and proceeding

to the highest. Record the response of the micro-coulometer to each calibration solution.

7.6.2 Prepare a method blank as described in Section 8.4. Subtract the value of the blank from each of the five calibration results, as described in Section 12.

7.6.3 Calibration factor (ratio of response to concentration): Using the blank subtracted results, compute the calibration factor at each calibration point, and compute the average calibration factor and the relative standard deviation (coefficient of variation; Cv) of the calibration factor over the calibration range.

7.6.4 Linearity: The Cv of the calibration factor shall be less than 20%; otherwise, the calibration shall be repeated after adjustment of the combustion/micro-coulometer system and/or preparation of fresh calibration standards.

8. QUALITY ASSURANCE/QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality assurance program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of matrix spike and matrix spike duplicate (MS/MSD) samples to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is demonstrated as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided that all performance specifications are met. Each time a modification is made to the method, the analyst is required to repeat the procedures in Sections 7 and 8.2 to demonstrate method performance.

8.1.3 The laboratory shall spike 10% of the samples with known concentrations of 2,4,6-trichlorophenol to monitor method performance and matrix interferences (interferences caused by the sample matrix). This test is described in Section 8.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits.

8.1.4 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of blanks are described in Section 8.4.

8.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the precision and recovery (PAR) standard that the analysis system is in control. These procedures are described in Section 11.

8.1.6 The laboratory shall perform quality control tests on the granular activated carbon. These procedures are described in Section 8.5.

8.1.7 Samples are analyzed in duplicate to demonstrate precision. These procedures are described in Section 8.6.

8.2 Initial precision and recovery (IPR): To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

8.2.1 Analyze four aliquots of the PAR standard (Section 6.12.4) and a method blank according to the procedures in Sections 8.4 and 10.

8.2.2 Using the blank-subtracted results of the set of four analyses, compute the average percent recovery (X) and the standard deviation of the percent recovery (s) for the results.

8.2.3 The average percent recovery shall be in the range of 77 to 108 μg/L and the standard deviation shall be less than 7 μg/L. If X and s meet these acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for recovery, system performance is unacceptable. In this case, correct the problem and repeat the test.

8.3 Matrix spikes: The laboratory shall spike a minimum of 10% of samples from a given matrix type (e.g., C-stage filtrate, produced water, treated effluent) in duplicate (MS/MSD). If only one sample from a given matrix type is analyzed, an additional two aliquots of that sample shall be spiked.

8.3.1 The concentration of the analytes spiked into the MS/MSD shall be determined as follows:

8.3.1.1 If, as in compliance monitoring, the concentration of OX is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration determined in Section 8.3.2, whichever concentration is higher.

8.3.1.2 If the concentration of OX is not being checked against a regulatory limit, the spike shall be at the concentration of the precision and recovery standard (PAR; Section 6.12.4) or at 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration is higher.

8.3.2 Analyze one sample out of each set of 10 samples from each site to determine the background concentration of AOX. If necessary, prepare a solution of 2,4,6-trichlorophenol appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike two additional sample aliquots with spiking solution and analyze them to determine the concentration after spiking.

8.3.2.1 Compute the percent recovery of each analyte in each aliquot:

$$\%\ Recovery = \frac{100\ (Found - Background)}{T}$$

where:

T is the true value of the spike

8.3.2.2 Compute the relative percent difference (RPD) between the two results (not between the two recoveries) as described in Section 12.4.

8.3.2.3 If the RPD is less than 20%, and the recoveries for the MS and MSD are within the range of 71 to 116%, the results are acceptable.

8.3.2.4 If the RPD is greater than 20%, analyze two aliquots of the precision and recovery standard (PAR).

8.3.2.4.1 If the RPD for the two aliquots of the PAR is greater than 20%, the analytical system is out of control. In this case, repair the problem and repeat the analysis of the sample set, including the MS/MSD.

8.3.2.4.2 If, however, the RPD for the two aliquots of the PAR is less than 20%, dilute the sample chosen for the MS/MSD by a factor of 10 and repeat the MS/MSD test. If the RPD is still greater than 20%, the result may not be reported for regulatory compliance purposes. In this case, choose another sample for the MS/MSD and repeat analysis of the sample set.

8.3.2.5 If the percent recovery for both the MS and MSD are less than 71% or greater than 116%, analyze the precision and recovery (PAR) standard.

8.3.2.5.1 If the recovery of the PAR is outside the 71 to 116% range, the analytical system is out of control. In this case, repair the problem and repeat the analysis of the sample set, including the MS/MSD.

8.3.2.5.2 If the recovery of the PAR is within the range of 71 to 116%, dilute the sample, MS, and MSD by a factor of 10 and re-analyze. If the results of the dilute analyses remain outside of the acceptable range, these results may not be reported for regulatory compliance purposes. In this case, choose another sample for the MS/MSD and repeat the analysis of the sample set.

8.4 Blanks: Reagent water blanks are analyzed to demonstrate freedom from contamination.

8.4.1 Analyze a reagent water blank with each set of samples prepared together. The blank must be analyzed immediately following calibration verification to demonstrate freedom from contamination and memory effects, and must include all details of the procedure to be followed when analyzing samples.

8.4.2 Prepare the reagent water blank from 100 mL of reagent water. If using the micro-column procedure, adsorb the method blank using two columns, as described in Section 10. Combust each column separately, as described in Section 10.

8.4.3 If the result from the blank from the shaker method or the sum of the results from two columns is more than 20 μg/L, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.5 Granular activated carbon (GAC) testing: Each batch of activated carbon is tested before use to ensure adequate quality. Use only GAC that meets the test criteria below.

8.5.1 Contamination test: Analyze a scoop of GAC. Reject carbon if the amount of OX exceeds 1 μg (25 μg Cl^-/g).

8.5.2 Inorganic chloride adsorption test: Attempt to adsorb NaCl from 100 mL of a solution containing 100 mg/L in reagent water. Wash with nitrate solution and analyze. The amount of halide should be less than 1 μg Cl^- larger than the blank. A larger amount indicates significant uptake of inorganic chloride by the carbon. Reject carbon if the 1 μg level is exceeded.

8.5.3 Carbon capacity test: Prepare an adsorption test standard solution in reagent water to contain 10 mg/L organic carbon (as humic acids or equivalent) and an organic halide concentration of 100 μg/L organo-chloride (from 2,4,6-trichlorophenol). Prepare a blank solution containing only the 10 mg organic carbon. Analyze 100-mL portions of these solutions. Subtract the result of the blank from the result of the halide spike, and compare the blank subtracted result to the true value of the spike. Recovery of the halide should be greater than 85%.

8.6 Samples that are being used for regulatory compliance purposes shall be analyzed in duplicate, at two different dilution levels.

8.6.1 The procedure for preparing duplicate sample aliquots is described in Section 10.5.

8.6.2 Calculate the RPD by following the same procedure described in Section 12.4.

8.6.3 If the RPD is greater than 20%, the analyses must be repeated.

8.6.4 If the RPD remains greater than 20%, the result may not be reported for regulatory compliance purposes.

8.7 The specifications contained in this method can be met if the apparatus used is calibrated properly and maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 11), and for initial (Section 8.2) and ongoing (Section 11) precision and recovery should be identical, so that the most precise results will be obtained.

8.8 Depending on specific program requirements, field duplicates may be collected to determine the precision of the sampling technique.

9. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

9.1 Sample preservation.

9.1.1 Residual chlorine: If the sample is known or suspected to contain free chlorine, the chlorine must be reduced to eliminate positive interference that may result from continued chlorination reactions. A knowledge of the process from which the sample is collected may be of value in determining whether dechlorination is necessary. Immediately after sampling, test for residual chlorine using the following method or an alternative EPA method (Reference 12):

9.1.1.1 Dissolve a few crystals of potassium iodide in the sample and add three to five drops of a 1% starch solution. A blue color indicates the presence of residual chlorine.

9.1.1.2 If residual chlorine is found, add 1 mL of sodium thiosulfate solution (Section 6.11) for each 2.5 ppm of free chlorine or until the blue color

disappears. Do not add an excess of sodium thiosulfate. Excess sodium thiosulfate may cause decomposition of a small fraction of the OX.

9.1.2 Acidification: Adjust the pH of all aqueous samples to <2 with nitric acid. Acidification inhibits biological activity and stabilizes chemical degradation, including possible dehalogenation reactions that may occur at high pH. Acidification is necessary to facilitate thorough adsorption.

9.1.3 Refrigeration: Maintain samples at a temperature of 0 to 4°C from time of collection until analysis.

9.2 Collect a minimum of 500 mL of sample in an amber glass bottle. This will provide a sufficient volume for all testing.

9.3 Analyze samples no less than three days nor more than six months after collection.

10. SAMPLE ANALYSIS

10.1 Sample dilution: Many samples will contain high concentrations of halide. If analyzed without dilution, the micro-coulometer can be overloaded, resulting in frequent cell cleaning and downtime. The following guidance is provided to assist in estimating dilution levels.

10.1.1 Paper and pulp mills that employ chlorine bleaching: Samples from pulp mills that use a chlorine bleaching process may overload the micro-coulometer. The following sample volumes are suggested for dilution:

Paper or Pulp Mill Stream	*Volume (mL)**
Evaporator condensate	100
process water	100
Pulp mill effluent	30
Pulp mill effluent	10
Combined mill effluent	5
Combined bleach effluent	1
C-stage filtrate	0.5
E-stage filtrate	0.5

* Dilution to final volume of 100 mL. All sample aliquots must be analyzed using a final volume of 100 mL (sample volume plus reagent water, as needed).

10.1.2 Sample dilution procedure.

10.1.2.1 Partially fill a precleaned 100-mL volumetric flask with pH <2 reagent water, allowing for the volume of sample to be added.

10.1.2.2 Mix sample thoroughly by tumbling or shaking vigorously.

10.1.2.3 Immediately withdraw the required aliquot using a pipet or micro-syringe.

NOTE: Because it will be necessary to rinse the pipet or micro-syringe (Section 10.1.2.5), it may be necessary to pre-calibrate the pipet or micro-syringe to assure that the exact volume desired will be delivered.

10.1.2.4 Dispense or inject the aliquot into the volumetric flask.

10.1.2.5 Rinse the pipet or syringe with small portions of reagent water and add to the flask.

10.1.2.6 Dilute to the mark with pH <2 reagent water.

10.1.3 All samples to be reported for regulatory compliance monitoring purposes must be analyzed in duplicate, at two dilution levels, as described in Section 10.5. Therefore, prepare a second dilution of the sample, using the procedure described in Section 10.1.2, but varying the volume of the aliquot in Section 10.1.2.3 by at least a factor of two.

10.1.4 Pulp and Paper in-process samples: The concentration of organic halide in in-process samples has been shown to be 20 to 30% greater using the micro-column adsorption technique than using the batch adsorption technique. For this reason, the micro-column technique shall be used for monitoring in-process samples. Examples of in-process samples include: Combined bleach plant effluents, C-stage filtrates, and E-stage filtrates.

10.2 Batch adsorption and filtration.

10.2.1 Place 100 mL (diluted if necessary) of sample that has been preserved as described in Section 9 into an Erlenmeyer flask.

10.2.2 Add 5 mL of nitrate stock solution to the sample aliquot.

10.2.3 Add one level scoop of activated carbon.

10.2.4 Shake the suspension for at least one hour in a mechanical shaker.

10.2.5 Filter the suspension through a polycarbonate membrane filter. Filter by suction until the liquid level reaches the top of the carbon.

10.2.6 Wash the inside surface of the filter funnel with approximately 25 mL of nitrate wash solution in several portions. After the level of the final wash reaches the top of the charcoal, filter by suction until the cake is barely dry. The time required for drying should be minimized to prevent exposure of the GAC to halogen vapors in the air, but should be sufficient to permit drying of the cake so that excess water is not introduced into the combustion apparatus. A drying time of approximately 10 seconds under vacuum has been shown to be effective for this operation.

10.2.7 Carefully remove the top of the filter holder, making sure that no carbon is lost. This operation is most successfully performed by removing the clamp, tilting the top of the filter holder (the funnel portion) to one side, and lifting upward.

10.2.8 Using a squeeze bottle or micro syringe, rapidly rinse the carbon from the inside of the filter holder onto the filter cake using small portions of wash solution. Allow the cake to dry under vacuum for no more than 10 seconds after the final rinse. Immediately turn the vacuum off.

10.2.9 Using the tweezers, carefully fold the polycarbonate filter in half, then in fourths, making sure that no carbon is lost.

10.3 Column adsorption.

10.3.1 Column preparation: Prepare a sufficient number of columns for one day's operation as follows:

10.3.1.1 In a glove box or area free from halide vapors, place a plug of Cerafelt into the end of a clean glass column.

10.3.1.2 Fill the glass column with one level scoop (approximately 40 mg) of granular activated carbon that has passed the quality control tests in Section 8.

10.3.1.3 Insert a Cerafelt plug into the open end of the column to hold the carbon in place.

10.3.1.4 Store the columns in a glass jar with PTFE lined screw-cap to prevent infiltration of halide vapors from the air.

10.3.2 Column setup.

10.3.2.1 Install two columns in series in the adsorption module.

10.3.2.2 If the sample is known or expected to contain particulates that could prevent free flow of sample through the micro-columns, a Cerafelt plug and a small wad of quartz wool are placed in the tubing ahead of the columns. If a measurement of the OX content of the particulates is desired, the Cerafelt plug and quartz wool can be washed with nitrate solution, placed in a combustion boat, and processed as a separate sample.

10.3.3 Adjusting column flow rate: Because the flow rate used to load the sample onto the columns can affect the ability of the GAC to adsorb the organic halides, the flow rate of the method blank is measured and the gas pressure used to process samples adjusted accordingly. The flow rate of the blank, composed of acidified reagent water containing no particulate matter, should be greater than the flow rate of any sample containing even small amounts of particulate matter.

10.3.3.1 Fill the sample reservoir with 100 mL ($\pm$0.5 mL) of reagent water that has been preserved and acidified as described in Section 9. Cap the reservoir.

10.3.3.2 Adjust the gas pressures per the manufacturer's instructions. Record the time required for the entire 100-mL volume of reagent water to pass through both columns. The flow rate must not exceed 3 mL/min over the duration of the time required to adsorb the 100-mL volume. If this flow rate is exceeded, adjust gas pressure, prepare another 100-mL blank, and repeat the adsorption.

10.3.3.3 Once the flow rate for the blank has been established, the same adsorption conditions and gas pressures must be applied to all subsequent samples during that 8-hour shift, or until another method blank is processed, whichever comes first. If the sample adsorption unit is disassembled or cleaned, the flow rate must be checked before processing additional samples.

10.3.3.4 Elute the pair of columns with 2 mL of nitrate wash solution.

10.3.3.5 Separate the columns and mark for subsequent analysis.

10.3.4 The adsorption of sample volumes is performed in a similar fashion. Fill the sample reservoir with 100-mL (± 5 mL) of sample that has been preserved as described in Section 9. All analyses must be performed with a 100-mL volume (sample volume plus reagent water, as needed) in order to maintain a flow rate no greater than that determined for the blank (see Section 10.3.3).

10.3.4.1 Use the same gas pressures for sample adsorption as is used for the blank.

10.3.4.2 Elute the columns with 2 mL of the nitrate wash solution.

10.3.4.3 Separate the columns and mark for subsequent analysis.

10.3.5 If it is desirable to make measurements at levels lower than can be achieved with a 100-mL sample, or if the instrument response of an undiluted sample is less than 3 times the instrument response of the blank (Section 12.6.3), a larger sample volume may be used. However, if a sample volume larger than 100 mL is used, the instrument must be recalibrated and all performance tests must be repeated, and all specifications in this method must be met, to demonstrate that reliable results can be obtained with the larger sample volume.

10.4 Combustion and titration.

10.4.1 Polycarbonate filter and GAC from batch adsorption.

10.4.1.1 Place the folded polycarbonate filter containing the GAC in a quartz combustion boat, close the airlock, and proceed with the automated sequence.

10.4.1.2 Record the signal from the micro-coulometer and determine the concentration of Cl^- from calibration data per Section 12.

10.4.2 Columns from column adsorption.

10.4.2.1 Using the push rod, push the carbon, the Cerafelt plug(s), and quartz wool (if used) from the first column into a combustion boat. Proceed with the automated sequence.

10.4.2.2 Record the signal from the micro-coulometer and determine the concentration of Cl^- for the first column from calibration data per Section 12.

10.4.2.3 Repeat the automated sequence with the second column.

10.4.2.4 Determine the extent of breakthrough of organic halides from the first column to the second column, as described in Section 12.

10.4.3 The two columns that are used for the method blank must be combusted separately, as is done for samples.

10.5 Duplicate sample analysis: All samples to be reported for regulatory compliance purposes must be analyzed in duplicate, and two dilution levels. This requirements applies to both the batch and column adsorption procedures.

10.5.1 Using the results from the analysis of one sample volume described in Section 10.4, and the procedure described in Section 10.1.2, prepare a second volume of sample at

a dilution level within either two times higher or two times lower than that used for the first analysis.

10.5.2 Adsorb the sample using the same technique (batch vs. column) used for the first sample volume. Combust the GAC from the second volume as described above, and calculate the results as described in Section 12. Compare the results of the two analyses as described in Section 12.4.

10.5.3 Duplicate analyses are not required for method blanks, as different dilution levels are not possible.

10.5.4 Duplicate analyses of the PAR standard used for calibration verification (see Section 11.2 below) are not required.

11. SYSTEM AND LABORATORY PERFORMANCE

11.1 At the beginning and end of each 8-hour shift during which analyses are performed, system performance and calibration are verified. Verification of system performance and calibration may be performed more frequently, if desired.

11.1.1 If performance and calibration are verified at the beginning and end of each shift (or more frequently), samples analyzed during that period are considered valid.

11.1.2 If performance and calibration are not verified at both the beginning and end of the shift (or more frequently), samples analyzed during that period must be reanalyzed.

11.1.3 If calibration is verified at the beginning of the shift, recalibration using the five standards described in Section 7.6 is not necessary; otherwise, the instrument must be recalibrated prior to analyzing samples (Section 7).

11.1.4 Cell maintenance and other changes to the analytical system that can affect system performance may not be performed during the 8-hour (or shorter) period.

11.2 Calibration verification and ongoing precision and recovery: Calibration and system performance are verified by the analysis of the 100 μg/L PAR standard.

11.2.1 Analyze the PAR standard (Section 6.12.4) and analyze a blank (Section 8.4) immediately thereafter at the beginning and end of each shift. Compute the concentration of organic halide in the PAR standard and in the blank, using the procedures in Section 12. The blank shall be less than 2 μg Cl^- (20 μg/L equivalent).

11.2.2 Subtract the result for the blank from the result of the PAR standard using the procedures in Section 12, and compute the percent recovery of the blank-subtracted PAR standard. The percent recovery shall be in the range of 71 to 116%.

11.2.3 If the recovery is within this range, the analytical process is in control and analysis of blanks and samples may proceed. If, however, the recovery is not within the acceptable range, the analytical process is not in control. In this event, correct the problem and repeat the ongoing precision and recovery test (Section 11.2.1), or recalibrate (Sections 7.5 through 7.6).

11.2.4 If the recovery is not within the acceptable range for the PAR standard analyzed at the end of the 8-hour shift, correct the problem, repeat the ongoing precision and

recovery test (Section 11.2), or recalibrate (Sections 7.5 through 7.6), and reanalyze the sample set that was analyzed during the 8-hour shift.

11.2.5 If the recovery is within the acceptable range at the end of the shift, and samples are to be analyzed during the next 8-hour shift, the end of shift verification may be used as the beginning of shift verification for the subsequent shift, provided the next 8-hour shift begins as the first shift ends.

11.3 Add results that pass the specification in Section 11.2.2 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for AOX by calculating the average percent recovery (R) and the standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

12. CALCULATIONS

12.1 Batch Adsorption Method: Calculate the blank-subtracted concentration of adsorbable organic halide in micrograms of chloride per liter detected in each sample using the following equation:

$$AOX\ (\mu g/L) = \frac{(C - B)}{V}$$

where:
C = *µg Cl^- from micro-coulometer for the sample*
B = *µg Cl^- from micro-coulometer for the blank*
V = *volume of sample in liters*

This calculation is performed for each of the two dilution levels analyzed for each sample.

12.2 Column Adsorption Method: Calculate the blank-subtracted concentration of adsorbable organic halide in micrograms of chloride per liter detected in each sample using the following equation:

$$AOX\ (\mu g/L) = \frac{[(C_1 + C_2) - (B_1 + B_2)]}{V}$$

where:
C_1 = *µg Cl^- from micro-coulometer for first column from the sample*
C_2 = *µg Cl^- from micro-coulometer for second column from the sample*
B_1 = *µg from micro-coulometer for first column from the blank*
B_2 = *µg Cl^- from micro-coulometer for second column from the blank*

12.3 Percent breakthrough: For each sample analyzed by the column method, calculate the percent breakthrough of halide from the first column to the second column, using the following equation:

$$\%\ Breakthrough = \frac{(C_2 - B_2)(100)}{[(C_1 - B_1) + (C_2 - B_2)]}$$

12.3.1 For samples to be reported for regulatory compliance purposes, the percent breakthrough must be less than or equal to 40% for both of the two analyses performed on each sample (see Section 10.5).

12.3.2 If the percent breakthrough in the second column exceeds 40%, dilute the affected sample further, maintaining the amount of halide at least three times higher than the level of blank, and reanalyze the sample. Ensure that the sample is also analyzed at a second level of dilution that is at least a factor of two different (and still higher than 3 times the blank).

12.4 Relative percent difference (RPD): Calculate the relative percent difference between the results of the two analyses of each sample, using the following equation:

$$RPD = \frac{100\ (AOX_1 - AOX_2)}{[(AOX_1 + AOX_2)/2]}$$

12.5 High concentrations of AOX: If the amount of chloride from either analysis exceeds the calibration range, dilute the sample and reanalyze, maintaining at least a factor of 2 difference in the dilution levels of the two portions of the sample used.

12.6 Low concentrations of AOX: The blank-subtracted final result from the batch procedure or the sum of the blank-subtracted results from the two carbon columns should be significantly above the level of the blank.

12.6.1 If the instrument response of a sample exceeds the instrument response of the blank by a factor of at least 3, the result is acceptable.

12.6.2 If the instrument response of a sample is less than 3 times the instrument response of the blank, and the sample has been diluted, analyze a less dilute aliquot of sample.

12.6.3 If the instrument response of an undiluted sample containing AOX above the minimum level is less than 3 times the instrument response of the blank, the result is suspect and may not be used for regulatory compliance purposes. In this case, find the cause of contamination, correct the problem, and reanalyze the sample under the corrected conditions.

12.7 Report results that meet all of the specifications in this method as the mean of the blank-subtracted values from Section 12.1 or 12.2 for the two analyses at different dilution levels, in μg/L of Cl^- (not as 2,4,6-trichlorophenol), to three significant figures. Report the RPD of the two analyses. For samples analyzed by the column procedure, also report the percent breakthrough.

13. METHOD PERFORMANCE

The specifications contained in this method are based on data from a single laboratory and from a large-scale study of the pulp and paper industry. These specifications will be updated as further data become available.

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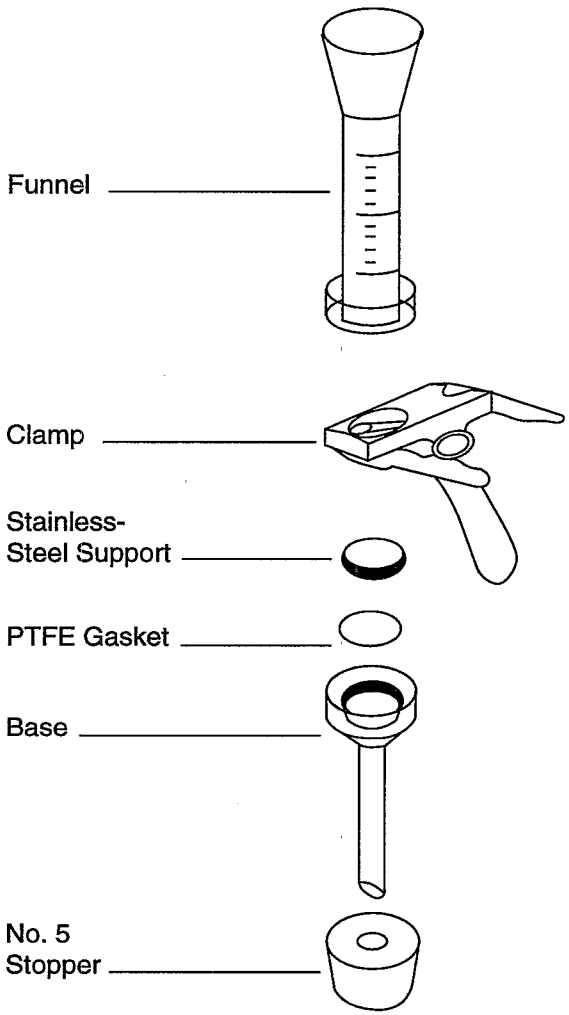

Figure 1. Filter Apparatus

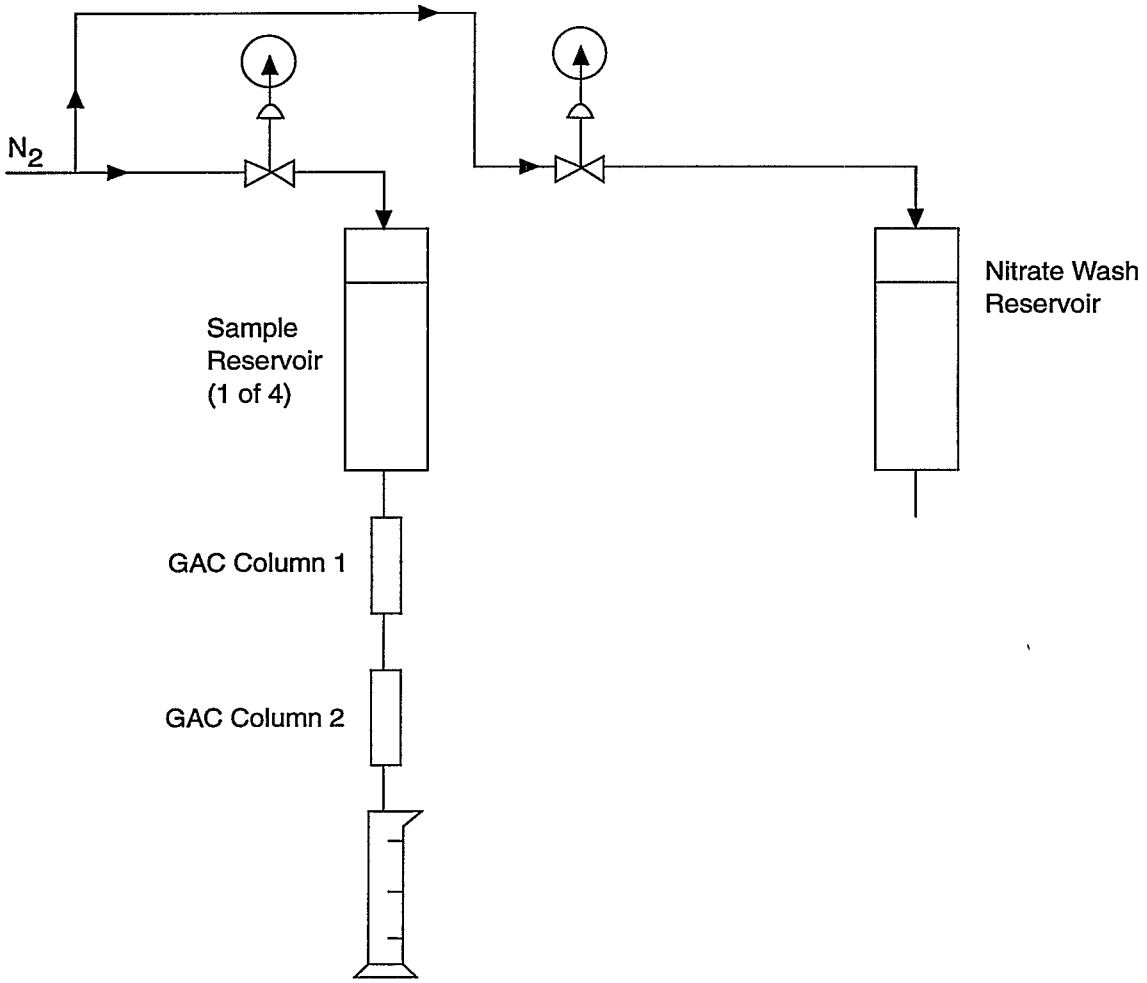

Figure 2. Schematic of the Column Adsorption System (from Reference 2)

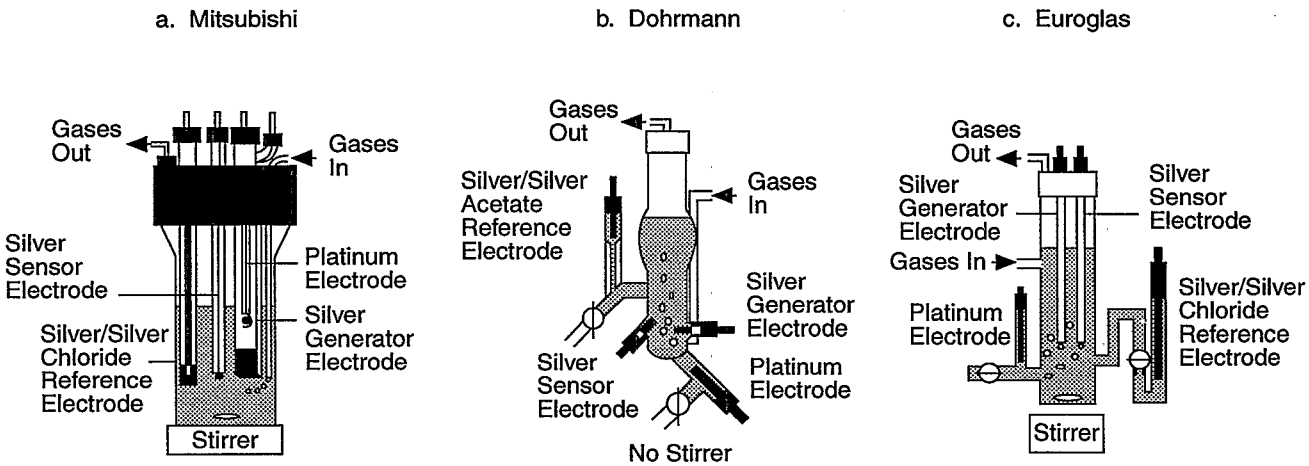

Figure 3. Microcoulometric Titration Cells (from Reference 7)

Method 1653

Chlorinated Phenolics in Wastewater by In Situ Acetylation and GCMS

1. SCOPE AND APPLICATION

1.1 This method is designed to determine chlorinated phenolics (chlorinated phenols, guaiacols, catechols, vanillins, syringaldehydes) and other compounds amenable to *in situ* acetylation, extraction, and analysis by capillary column gas chromatography/mass spectrometry (GCMS). The method is based on existing methods for determination of chlorophenolics in pulp and paper industry wastewaters (References 1 and 2).

1.2 The chemical compounds listed in Table 1 may be determined in waters and specifically in in-process streams and wastewaters associated with the paper and pulp industry. The method is designed to meet the survey requirements of the Environmental Protection Agency (EPA).

1.3 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantity that can be detected with no interferences present.

1.4 The GCMS portions of this method are for use only by analysts experienced with GCMS or under the close supervision of such qualified persons. Laboratories unfamiliar with analyses of environmental samples by GCMS should run the performance tests in Reference 3 before beginning.

1.5 Any modification of the method beyond those expressly permitted is subject to the application and approval of alternative test procedures under 40 *CFR* Parts 134 and 135.

2. SUMMARY OF METHOD

2.1 A 1000-mL aliquot of water is adjusted to neutral pH. Potassium carbonate buffer is added and the pH is raised to between 9 and 11.5. Stable isotopically labeled analogs of the compounds of interest and an internal standard are added to the solution. The chlorophenolics are converted *in situ* to the acetates by the addition of acetic anhydride.

After acetylation, the solution is extracted with hexane. The hexane is concentrated to a final volume of 0.5 mL, an instrument internal standard is added, and an aliquot of the concentrated extract is injected into the gas chromatograph (GC). The compounds are separated by GC and detected by a mass spectrometer (MS). The labeled compounds and internal standard serve to correct the variability of the analytical technique.

2.2 Identification of a pollutant (qualitative analysis) is performed by comparing the relative retention time and mass spectrum to that of an authentic standard. A compound is identified when its relative retention time and mass spectrum agree.

2.3 Quantitative analysis is performed in one of two ways by GCMS using extracted ion-current profile (EICP) areas: (1) For those compounds listed in Table 1 for which standards and labeled analogs are available, the GCMS system is calibrated and the compound concentration is determined using an isotope dilution technique; (2) for those compounds listed in Table 1

for which authentic standards but no labeled compounds are available, the GCMS system is calibrated and the compound concentration is determined using an internal standard technique.

2.4 Quality is assured through reproducible calibration and testing of the extraction and GCMS systems.

3. CONTAMINATION, INTERFERENCES, AND ANALYTE DEGRADATION

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms and spectra. All materials 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 8-hour shift, to a maximum of 20). Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Glassware and, where possible, reagents are cleaned by solvent rinse and baking at 450°C for a minimum of 1 hour.

3.2 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Industry experience suggests (Reference 1) that high levels of non-chlorinated phenols may cause poor recovery of the compounds of interest, particularly in samples collected in the vicinity of a source of creosote, such as a wood-preserving plant.

3.3 The internal standard, 3,4,5-trichlorophenol, has been reported (Reference 1) to be an anaerobic degradation product of 2,3,4,5-tetrachlorophenol and/or pentachlorophenol. When an interference with this compound occurs, labeled pentachlorophenol may be used as an alternative internal standard.

3.4 Blank contamination by pentachlorophenol has been reported (Reference 1) to be traceable to potassium carbonate; it has also been reported that this contamination may be removed by baking overnight at 400 to 500°C.

3.5 Catechols are susceptible to degradation by active sites on injection port liners and columns, and are subject to oxidation to the corresponding chloro-*o*-benzoquinones (Reference 2). A small amount of ascorbic acid may be added to samples to prevent auto-oxidation (Reference 2; also see Section 10.1.7).

4. SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. 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 data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 4 through 6.

4.2 Samples may contain high concentrations of toxic compounds, and should be handled with gloves and a hood opened to prevent exposure.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps.

5.1.1.1 Sample bottle, amber glass, 1000-mL minimum, with screw-cap. If amber bottles are not available, samples shall be protected from light.

5.1.1.2 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.1.3 Cleaning bottles: Detergent water wash, cap with aluminum foil, and bake at 450°C for a minimum of 1 hour before use.

5.1.1.4 Cleaning liners: Detergent water wash, reagent water (Section 6.5.1) and solvent rinse, and bake at approximately 200°C for a minimum of 1 hour prior to use.

5.1.1.5 Bottles and liners must be lot-certified to be free of chlorophenolics by running blanks according to this method. If blanks from bottles and/or liners without cleaning or with fewer cleaning steps show no detectable chlorophenolics, the bottle and liner cleaning steps that do not eliminate chlorophenolics may be omitted.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. 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 (Section 6.4) to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Extraction apparatus.

5.2.1 Bottle or beaker: 1500- to 2000-mL capacity.

5.2.2 Separatory funnel: 500- to 2000-mL, glass, with PTFE stopcock.

5.2.3 Magnetic stirrer: Corning Model 320, or equivalent, with stirring bar.

5.3 Polyethylene gloves: For handling samples and extraction equipment (Fisher 11-394-110-B, or equivalent).

5.4 Graduated cylinders: 1000-mL, 100-mL, and 10-mL nominal.

5.5 Centrifuge: Capable of accepting 50-mL centrifuge tubes and achieving 3000 RPM.

5.5.1 Centrifuge tubes.

5.5.1.1 35-mL nominal, with PTFE-lined screw-cap.

5.5.1.2 15-mL nominal, conical graduated, with ground-glass stopper.

5.6 Concentration apparatus.

5.6.1 Kuderna-Danish (K-D) concentrator tube: 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.6.2 Kuderna-Danish (K-D) evaporation flask: 1000-mL (Kontes K-570001-1000, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).

5.6.3 Snyder column: Three-ball macro (Kontes K-503000-0232, or equivalent).

5.6.4 Snyder column: Two-ball micro (Kontes K-469002-0219, or equivalent).

5.6.5 Boiling chips: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for a minimum of 1 hour.

5.6.6 Nitrogen evaporation apparatus: Equipped with a water bath controlled at 35 to 40°C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood. This device may be used in place of the micro-Snyder column concentrator in Section 5.6.4 above.

5.7 Water bath: Heated, with concentric ring cover, capable of temperature control (± 2°C), installed in a fume hood.

5.8 Sample vials: Amber glass, 1- to 3-mL with PTFE-lined screw-cap.

5.9 Balances.

5.9.1 Analytical: Capable of weighing 0.1 mg.

5.9.2 Top loading: Capable of weighing 10 mg.

5.10 pH meter.

5.11 Gas chromatograph: Shall have splitless or on-column injection port for capillary column, temperature program with 50°C hold, and shall meet all of the performance specifications in Section 12.

5.12 Gas chromatographic column: 30 (±5 m) × 0.25 (±0.02 mm) I.D. × 0.25 micron, 5% phenyl, 94% methyl, 1% vinyl silicone bonded-phase fused-silica capillary column (J & W DB-5, or equivalent).

5.13 Mass spectrometer: 70 eV electron impact ionization, shall repetitively scan from 35 to 450 amu in 0.95 to 1.00 second, and shall produce a unit resolution (valleys between m/z 441–442 less than 10% of the height of the 441 peak), background-corrected mass spectrum from 50 ng decafluorotriphenylphosphine (DFTPP) introduced through the GC inlet. The spectrum shall meet the mass-intensity criteria in Table 3 (Reference 7). The mass spectrometer shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beams. All portions of the column which connect the GC to the ion source shall remain at or above the column temperature during analysis to preclude condensation of less volatile compounds.

5.14 Data system: Shall collect and record MS data, store mass-intensity data in spectral libraries, process GCMS data, generate reports, and shall compute and record response factors.

5.14.1 Data acquisition: Mass spectra shall be collected continuously throughout the analysis and stored on a mass storage device.

5.14.2 Mass spectral libraries: User-created libraries containing mass spectra obtained from analysis of authentic standards shall be employed to reverse search GCMS runs for the compounds of interest (Section 7.2).

5.14.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each GCMS analysis. Software routines shall be

employed to compute retention times, and to compute peak areas at the m/z's specified (Table 4). Displays of spectra, mass chromatograms, and library comparisons are required to verify results.

5.14.4 Response factors and multi-point calibrations: The data system shall be used to record and maintain lists of response factors (response ratios for isotope dilution) and multi-point calibration curves (Section 7). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 12.4) performance shall be computed and maintained.

6. REAGENTS AND STANDARDS

6.1 Reagents for adjusting sample pH.

6.1.1 Sodium hydroxide: Reagent grade, 6 N in reagent water.

6.1.2 Sulfuric acid: Reagent grade, 6 N in reagent water.

6.2 Reagents for sample preservation.

6.2.1 Sodium thiosulfate ($Na_2S_2O_3$) solution (1 N): Weight 79 g $Na_2S_2O_3$ in a 1-L volumetric flask and dilute to the mark with reagent water.

6.2.2 Ascorbic acid solution: Prepare a solution of ascorbic acid in reagent water at a concentration of 0.1 g/mL. This solution must be prepared fresh each day on which derivatizations will be performed. Therefore, do not prepare more than will likely be used that day. (A 50-mL volume will be sufficient for ten analyses).

6.3 Solvents: Hexane, acetone, and methanol. Distilled in glass (Burdick and Jackson, or equivalent).

6.4 Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method.

6.5 Reagents for derivatization.

6.5.1 Potassium carbonate (K_2CO_3).

6.5.1.1 Purification: Spread in a shallow baking dish, heat overnight at 400 to 500°C.

6.5.1.2 Solution: Dissolve 150 g purified K_2CO_3 in 250 mL reagent water.

6.5.2 Acetic anhydride: Redistilled reagent grade.

6.6 Analytical standards.

6.6.1 Derivatization: Because the chlorinated phenolics are determined as their acetate derivatives after *in situ* acetylation, the method requires that the calibration standards be prepared by spiking the underivatized materials into reagent water and carrying the spiked reagent water aliquot through the entire derivatization and extraction procedure that is applied to the field samples.

6.6.2 Standard solutions: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or 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 standard. When not being used, standards are stored in the dark at −20 to −10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use.

6.6.3 If the chemical purity of any standard does not meet the 98% requirement above, the laboratory must correct all calculations, calibrations, etc., for the difference in purity.

6.7 Preparation of stock solutions: Prepare in acetone per the steps below. Observe the safety precautions in Section 4.

6.7.1 Dissolve an appropriate amount of assayed reference material in a suitable solvent. For example, weigh 50 mg (±0.1 mg) of pentachlorophenol in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with acetone. After the pentachlorophenol is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.

6.7.2 Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards and shall be replaced after six months, or sooner if comparison with quality control check standards indicates a change in concentration.

6.8 Labeled compound spiking solution: From stock solutions prepared as above, or from mixtures, prepare the spiking solution in acetone at the following concentrations:

4-Chloroguaiacol-$^{13}C_6$	12.5	µg/mL
2,4-Dichlorophenol-d_3	25.0	µg/mL
5-Chlorovanillin-$^{13}C_6$	25.0	µg/mL
4,5-Dichlorocatechol-$^{13}C_6$	25.0	µg/mL
4,5,6-Trichloroguaiacol-$^{13}C_6$	25.0	µg/mL
Pentachlorophenol-$^{13}C_6$	50.0	µg/mL
Tetrachloroguaiacol-$^{13}C_6$	50.0	µg/mL
Tetrachlorocatechol-$^{13}C_6$	50.0	µg/mL
3,4,5-Trichlorophenol	50.0	µg/mL

Prepare all standards containing chlorovanillins in acetone, as these compounds are subject to degradation in methanol.

6.9 Secondary standard for calibration: Using stock solutions (Section 6.7), prepare a secondary standard containing the compounds in Table 1 in acetone. The monochlorinated phenol, guaiacol, and catechol are included at a concentration of 25 µg/mL; the trichlorinated catechols, tetrachlorinated guaiacol and catechol, pentachlorophenol, 5,6-dichlorovanillin, and 2,6-dichlorosyringaldehyde are included at a concentration of 100 µg/mL, and the remaining compounds are included at a concentration of 50 µg/mL. This solution is the nominal 50 µg/mL calibration solution. Final extract concentrations for this solution are shown in the column titled "Test Conc. (µg/mL)" in Table 5.

6.10 Instrument internal standard (IIS): Prepare a solution of 2,2′-difluorobiphenyl (DFB) at a concentration of 5.0 mg/mL in acetone.

6.11 DFTPP solution: Prepare a solution of DFTPP at 50 μg/mL in acetone.

6.12 Solutions for obtaining authentic mass spectra (Section 7.2): Prepare mixtures of compounds at concentrations which will assure authentic spectra are obtained for storage in libraries.

6.13 Preparation of calibration solutions.

6.13.1 Into five 1000-mL aliquots of reagent water, spike 50, 100, 200, 500 and 1000 μL of the solution in Section 6.9. Spike 1.00 mL of the labeled compound spiking solution (Section 6.8) into each of the five aliquots.

6.13.2 Using the procedure in Section 10, derivatize and extract each solution, and concentrate the extract to a final volume of 0.50 mL. This will produce calibration solutions of nominal 5, 10, 20, 50, and 100 μg/mL of the chlorophenolics and a constant concentration of each labeled compound (though levels vary by labeled compound, they are constant across the five calibration solutions), and 50 μg/mL of the 3,4,5-trichlorophenol SMIS (assuming 100% derivatization and recovery). As noted in Section 10.1.7, ascorbic acid is added to all samples of final effluents to stabilize chlorocatechols, but is not added to samples of pulp and paper in-process wastewaters. Therefore, it is necessary to prepare separate sets of five initial calibration standards with and without the addition of ascorbic acid. Also, in the event that the laboratory is extracting final effluent samples by both the stir-bar and separatory funnel procedures (see Section 10.3), initial calibration standards should be prepared by both methods.

6.13.3 Spike each 0.5-mL extract with 5 μL of the 2,2′-difluorobiphenyl IIS (Section 6.10).

6.13.4 These solutions permit the relative response (labeled to unlabeled) and the response factor to be measured as a function of concentration (Sections 7.4 through 7.5).

6.13.5 The nominal 50 μg/mL standard may also be used as a calibration verification standard (see Section 12.3).

6.14 Ongoing precision and recovery (OPR) standard: Used for determination of initial (Section 8.2) and ongoing (Section 12.3) precision and recovery. This solution is prepared by spiking 500 μL of the secondary calibration standard (Section 6.9) and 1 mL of the labeled compound spiking solution (Section 6.8) into 1000 mL of reagent water.

6.15 Stability of solutions: All standard solutions (Sections 6.8 through 6.14) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area at the quantitation m/z relative to the DFB internal standard remains within ±15% of the area obtained in the initial analysis of the standard.

7. CALIBRATION

7.1 Assemble the GCMS and establish the operating conditions in Section 11. Analyze standards per the procedure in Section 11 to demonstrate that the analytical system meets the minimum levels in Table 2, and the mass-intensity criteria in Table 3 for 50 ng DFTPP.

7.2 Mass-spectral libraries: Detection and identification of compounds of interest are dependent upon spectra stored in user-created libraries.

7.2.1 Obtain a mass spectrum of the acetyl derivative of each chlorophenolic compound (pollutant, labeled compound, and the sample matrix internal standard) 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. That only a single compound is present is determined by examination of the spectrum. Fragments not attributable to the compound under study indicate the presence of an interfering compound.

7.2.2 Adjust the analytical conditions and scan rate (for this test only) to produce an undistorted spectrum at the GC peak maximum. An undistorted spectrum will usually be obtained if five complete spectra are collected across the upper half of the GC peak. Software algorithms designed to "enhance" the spectrum may eliminate distortion, but may also eliminate authentic m/z's or introduce other distortion.

7.2.3 The authentic reference spectrum is obtained under DFTPP tuning conditions (Section 7.1 and Table 3) to normalize it to spectra from other instruments.

7.2.4 The spectrum is edited by removing all peaks in the m/z 35 to 45 range, and saving the five most intense mass spectral peaks and all other mass spectral peaks greater than 10% of the base peak (excluding the peaks in the m/z 35 to 45 range). The spectrum may be further edited to remove common interfering masses. The spectrum obtained is stored for reverse search and for compound confirmation.

7.3 Analytical range and minimum level.

7.3.1 Demonstrate that 50 ng of 2,2'-difluorobiphenyl produces an area at m/z 190 approximately one-fourth that required to exceed the linear range of the system. The exact value must be determined by experience for each instrument. It is used to match the calibration range of the instrument to the analytical range and detection limits required, and to diagnose instrument sensitivity problems (Section 15.3). The nominal 50 μg/mL calibration standard (Section 6.13) can be used to demonstrate this performance.

7.3.2 Demonstrate that the chlorophenolics are detectable at the minimum level (per all criteria in Section 13). The nominal 5 μg/mL calibration standard (Section 6.13) can be used to demonstrate this performance.

7.4 Calibration with isotope dilution: Isotope dilution is used when (1) labeled compounds are available, (2) interferences do not preclude its use, and (3) the quantitation m/z (Table 4) extracted ion-current profile (EICP) area for the compound is in the calibration range. Alternative labeled compounds and quantitation m/z's may be used based on availability. If any of the above conditions preclude isotope dilution, the internal standard calibration method (Section 7.5) is used.

7.4.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (pollutant to labeled) vs. concentration in standard solutions is plotted or computed using a linear regression. The example in Figure 1 shows a calibration curve for phenol using phenol-d5 as the isotopic diluent. Also shown are the $\pm$10% error limits (dotted lines). Relative response (RR) is determined according to the procedures described below. A minimum of five data points are employed for calibration.

7.4.2 The relative response of a pollutant to its labeled analog is determined from isotope ratio values computed from acquired data. Three isotope ratios are used in this process:

R_x = *the isotope ration measured for the pure pollutant.*
R_y = *the isotope ration measured for the labeled compound.*
R_m = *the isotope ration of an analytical mixture of pollutant and labeled compounds.*

The m/z's are selected such that $R_x > R_y$. If R_m is not between $2R_y$ and $0.5R_x$, the method does not apply and the sample is analyzed by the internal standard method.

7.4.3 Capillary columns sometimes separate the pollutant-labeled pair when deuterium labeled compounds are used, with the labeled compound eluted first (Figure 2). For this case,

$$R_x = \left[\frac{area\ m_1/z}{1}\right], \text{ at the retention time of the pollutant } (RT_2).$$

$$R_y = \frac{1}{[area\ m_2/z]}, \text{ at the retention time of the labeled compound } (RT_1).$$

$$R_m = \frac{[area\ at\ m_1/z\ (at\ RT_2)]}{[area\ at\ m_2/z\ (at\ RT_1)]}, \text{ as measured in the mixture of the pollutant and labeled compounds (Figure 2), and } RR = R_m.$$

7.4.4 When the pollutant-labeled pair is not separated (as occurs with carbon-13-labeled compounds), or when another labeled compound with interfering spectral masses overlaps the pollutant (a case which can occur with isomeric compounds), it is necessary to determine the contributions of the pollutant and labeled compound to the respective EICP areas. If the peaks are separated well enough to permit the data system or operator to remove the contributions of the compounds to each other, the equations in Section 7.4.3 apply. This usually occurs when the height of the valley between the two GC peaks at the same m/z is less than 70 to 90% of the height of the shorter of the two peaks. If significant GC and spectral overlap occur, RR is computed using the following equation:

$$RR = \frac{(R_y - R_m)(R_x + 1)}{(R_m - R_x)(R_y + 1)}$$

Where:

R_x *is measured as shown in figure* 3*A*,
R_y *is measured as shown in figure* 3*B*,
R_m *is measured as shown in figure* 3*C*.

For example, R_x = 46100/4780 = 9.644; R_y = 2650/43600 = 0.0608; R_m = 49200/48300 = 1.1019; thus, RR = 1.114.

7.4.5 To calibrate the analytical system by isotope dilution, analyze a 1-μL aliquot of each of the calibration standards (Section 6.13) using the procedure in Section 11. Compute the RR at each concentration.

7.4.6 Linearity: If the ratio of relative response to concentration for any compound is constant (less than 20% coefficient of variation) over the five-point calibration range, an averaged relative response/concentration ratio may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

7.5 Calibration by internal standard: The method contains two types of internal standards, the sample matrix internal standard (SMIS) and the instrument internal standard (IIS), and they are used for different quantitative purposes. The 3,4,5-trichlorophenol sample matrix internal standard (SMIS) is used for measurement of all pollutants with no labeled analog and when the criteria for isotope dilution (Section 7.4) cannot be met. The 2,2′-difluorobiphenyl instrument internal standard (IIS) is used for determination of the labeled compounds and the SMIS. The results are used for intralaboratory statistics (Sections 8.4 and 12.4).

7.5.1 Response factors: Calibration requires the determination of response factors (RF) for both the pollutants with no labeled analog and for the labeled compounds and the SMIS. The response factors are defined by the following equation:

$$RF = \frac{(A_s \times C_{is})}{(A_{is} \times C_s)}$$

Where:
A_s = *the area of the chracteristic mass for the compound in the daily standard*
A_{is} = *the area of the characteristic mass for the internal standard*
C_{is} = *the concentration of the internal standard* (*μg/mL*)
C_s = *is the concentration of the compound in the calibration standard* (*μg/mL*)

When this equation is used to determine the response factors for pollutant compounds without labeled analogs, use the area (A_{is}) and concentration (C_{is}) of 3,4,5-trichlorophenol (SMIS) as the internal standard. When this equation is used to determine the response factors for the labeled analogs and the SMIS, use the area (A_{is}) and concentration (C_{is}) of 2,2′-difluorobiphenyl as the internal standard.

7.5.2 The response factor is determined for at least five concentrations appropriate to the response of each compound (Section 6.13); nominally, 5, 10, 20, 50, and 100 μg/mL. The amount of SMIS added to each solution is the same (50 μg/mL) so that C_{is} remains constant. Likewise, the concentration of IIS is constant in each solution. The RF is plotted versus concentration for each compound in the standard (C_s) to produce a calibration curve.

7.5.3 Linearity: If the response factor (RF) for any compound is constant (less than 35% coefficient of variation) over the five-point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point range.

7.6 Combined calibration: By using calibration solutions (Section 6.13) containing the pollutants, labeled compounds, and the internal standards, a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods. These curves are verified each shift (Section 12) by analyzing the OPR standard, or an optional calibration verification (CALVER) standard. Recalibration is required only if OPR criteria (Section 12.4 and Table 5) cannot be met.

8. QUALITY ASSURANCE/QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 8). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance specifications are met. Each time a modification is made to the method, the analyst is required to repeat the procedures in Sections 7.3.1 and 8.2 to demonstrate method performance.

8.1.3 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.5.

8.1.4 The laboratory shall spike all samples with labeled compounds and the sample matrix internal standard (SMIS) to monitor method performance. This test is described in Section 8.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits (Section 15).

8.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery standard (Section 6.14) that the analysis system is in control. These procedures are described in Section 12.

8.1.6 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.2 Initial precision and recovery (IPR): To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

8.2.1 Derivatize, extract, concentrate, and analyze four 1000-mL aliquots of the ongoing precision and recovery standard (OPR; Section 6.14) according to the procedure in Section 10. Separate sets of IPR aliquots must be prepared with the addition of ascorbic acid and without.

8.2.2 Using results of the four analyses, compute the average recovery (X) in μg/mL and the standard deviation of the recovery (s) in μg/mL for each compound, by isotope dilution for pollutants with a labeled analog, and by internal standard for pollutants with no labeled analog and for the labeled compounds and the SMIS.

8.2.3 For each compound, compare s and X with the corresponding limits for initial precision and recovery in Table 5. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for recovery, system performance is unacceptable for that compound. In this event, correct the problem and repeat the test (Section 8.2.1).

8.3 The laboratory shall spike all samples with labeled compounds and the sample matrix internal standard (SMIS) to assess method performance on the sample matrix.

8.3.1 Analyze each sample according to the method beginning in Section 10.

8.3.2 Compute the percent recovery (P) of the labeled compounds and the SMIS using the internal standard method (Section 7.5) with 2,2′-difluorobiphenyl as the reference compound.

8.3.3 Compare the labeled compound and SMIS recovery for each compound with the corresponding limits in Table 5. If the recovery of any compound falls outside its warning limit, method performance is unacceptable for that compound in that sample. Therefore, the sample is complex. The sample is diluted and reanalyzed per Section 15.

8.4 As part of the QA program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five samples for which the labeled compounds pass the tests in Section 8.3, compute the average percent recovery (P) and the standard deviation of the percent recovery (sp) for the labeled compounds only. Express the accuracy assessment as a percent recovery interval from P − 2sp to P + 2sp for each matrix. For example, if P = 90% and sp = 10%, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment for each compound on a regular basis (e.g., after each five to ten new accuracy measurements).

8.5 Blanks: Reagent water blanks are analyzed to demonstrate freedom from contamination.

8.5.1 Extract and concentrate a 1000-mL reagent water blank with each sample batch (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Blanks associated with samples to which ascorbic acid is added must be prepared with ascorbic acid. Blanks associated with samples to which ascorbic acid is not added must be prepared without ascorbic acid. Analyze the blank immediately after analysis of the OPR (Section 6.14) to demonstrate freedom from contamination.

8.5.2 If any of the compounds of interest (Table 1) or any potentially interfering compound is found in an aqueous blank at greater than 5 μg/L (assuming a response factor of 1 relative to the sample matrix internal standard for compounds not listed in Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.6 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7) and for initial (Section 8.2) and ongoing (Section 12) precision and recovery should be identical, so that the most precise results will be obtained. The GCMS instrument in particular will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of chlorophenolics by this method.

8.7 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Collect samples in glass containers (Section 5.1) following conventional sampling practices (Reference 9). Aqueous samples are collected in refrigerated bottles using automatic sampling equipment.

9.2 Sample preservation.

9.2.1 Residual chlorine: If the sample contains residual chlorine, the chlorine must be reduced to eliminate positive interference resulting from continued chlorination reactions. Immediately after sampling, test for residual chlorine using the following method or an alternative EPA method (Reference 10):

9.2.1.1 Dissolve a few crystals of potassium iodide in the sample and add three to five drops of a 1% starch solution. A blue color indicates the presence of residual chlorine.

9.2.1.2 If residual chlorine is found, add 1 mL of sodium thiosulfate solution (Section 6.2.1) for each 2.5 ppm of free chlorine or until the blue color disappears.

9.2.2 Acidification: Adjust pH of all aqueous samples to <2 with sulfuric acid (Section 6.1.2). Failure to acidify samples may result in positive interferences from continued chlorination reactions.

9.2.3 Refrigeration: Maintain sample temperature at 0 to 4°C from time of collection until extraction, and maintain extracts at a temperature of 0 to 4°C from time of extraction until analysis.

9.3 Collect a minimum of 2000 mL of sample. This will provide a sufficient amount for all testing. Smaller amounts may be collected if the stream is known to contain high levels of chlorophenolics.

9.4 All samples must be acetylated and extracted within 30 days of collection, and must be analyzed within 30 days of acetylation.

10. SAMPLE DERIVATIZATION, EXTRACTION, AND CONCENTRATION

The procedure described in this section uses a stir-bar in a beaker for the derivatization. The extraction procedures applied to samples depend on the type of sample being analyzed. Extraction of samples from in-process wastewaters is performed using a separatory funnel procedure. All

calibrations, IPR, OPR, and blank analyses associated with in-process wastewater samples must be performed by the separatory funnel procedure.

Extraction of samples of final effluents and raw water may be performed using either the stir-bar procedure or the traditional separatory funnel procedure. However, all calibrations, IPR, OPR, blank, and sample analyses must be performed using the same procedure. Both procedures are described below.

10.1 Preparation of all sample types for stir-bar derivatization.

10.1.1 Allow sample to warm to room temperature.

10.1.2 Immediately prior to measuring, shake sample vigorously to insure homogeneity.

10.1.3 Measure 1000 mL (±10 mL) of sample into a clean 2000-mL beaker. Label the beaker with the sample number.

10.1.4 Dilute aliquot(s).

10.1.4.1 Complex samples: For samples that are expected to be difficult to derivatize, concentrate, or are expected to overload the GC column or mass spectrometer, measure an additional 100 mL (±1 mL) into a clean 2000-mL beaker and dilute to a final volume of 1000-mL (±50 mL) with reagent water. Label with the sample number and as the dilute aliquot. However, to ensure adequate sensitivity, a 1000-mL aliquot must always be prepared and analyzed.

10.1.4.2 Pulp and paper industry samples: For in-process streams such as E-stage and C-stage filtrates and other in-process wastewaters, it may be necessary to prepare an aliquot at an additional level of dilution. In this case, dilute 10 mL (±0.1 mL) of sample to 1000-mL (±50 mL). However, here again, to ensure adequate sensitivity, a 1000-mL aliquot must always be prepared and analyzed.

10.1.5 QC aliquots: For a batch of samples of the same type to be extracted at the same time (to a maximum of 20), place two 1000-mL (±10 mL) aliquots of reagent water in clean 2000-mL beakers. Label one beaker as the blank and the other as the ongoing precision and recovery (OPR) aliquot.

Because final effluent samples are treated with ascorbic acid and in-process wastewater samples are not (see Section 10.1.7), prepare an OPR aliquot and a blank for the final effluent and a separate pair for the in-process samples. Treat these QC aliquots in the same fashion as the associated samples, adding ascorbic acid to the pair associated with the final effluents, and not adding ascorbic acid to the pair associated with the in-process samples.

10.1.6 Adjust the pH of the sample aliquots to between 7.0 and 7.1. For calibration standards, IPR and OPR aliquots and blanks, which are prepared in reagent water and therefore have little inherent buffering capacity, adjust the pH to between 6.5 and 7.5.

10.1.7 Ascorbic acid: Added to stabilize chlorocatechols. However, for pulp and paper industry in-process streams and other in-process wastewaters, the addition of ascorbic acid may convert chloro-*o*-quinones to catechols if these quinones are present.

Separate calibration curves must be prepared with and without the addition of ascorbic acid (Section 6.13.2).

10.1.7.1 Spike 5 to 6 mL of the ascorbic acid solution (Section 6.2.2) into each final effluent sample, and the associated calibration standards, IPR and OPR aliquots, and blank.

10.1.7.2 For paper and pulp industry C-stage filtrates, E-stage filtrates, and untreated effluents, omit the ascorbic acid to prevent the conversion of chloro-*o*-quinones to catechols. Prepare calibration standards, IPR and OPR aliquots, and blanks associated with these samples without ascorbic acid as well.

10.1.8 Spike 1000 µL of the labeled compound spiking solution (Section 6.8) and into the sample and QC aliquots.

10.1.9 Spike 500 µL of the nominal 50 µg/mL calibration solution (Section 6.9) into the OPR aliquot.

10.1.10 Equilibrate all sample and QC solutions for approximately 1 hour, with occasional stirring.

10.2 Derivatization: Because derivatization must proceed rapidly, particularly upon the addition of the acetic anhydride, it is necessary to work with one sample at a time until the derivatization step (Section 10.2.3) is complete. It may is also be efficient to extract the derivatization products immediately.

10.2.1 Place a beaker containing a sample or QC aliquot on the magnetic stirrer in a fume hood, drop a clean stirring bar into the beaker, and increase the speed of the stirring bar until the vortex is drawn to the bottom of the beaker.

10.2.2 Add 25 to 26 mL of K_2CO_3 buffer to the sample or QC aliquot.

10.2.3 Immediately add approximately 25 mL of acetic anhydride and stir for 3 to 5 minutes to complete the derivatization.

10.3 Extraction: Two procedures are described below for the extraction of derivatized samples. The choice of extraction procedure will depend on the sample type. For final effluent samples, either of two procedures may be utilized for extraction of derivatized samples. For samples of in-process wastewaters, the separatory funnel extraction procedure must be used.

NOTE: Whichever procedure is employed, the same extraction procedure must be used for calibration standards, IPR aliquots, OPR aliquots, blanks, and the associated field samples.

10.3.1 Stir-bar extraction of final effluents.

10.3.1.1 Add 200 mL (±20 mL) of hexane to the beaker and stir for 3 to 5 minutes, drawing the vortex to the bottom of the beaker.

10.3.1.2 Stop the stirring and drain the hexane and a portion of the water into a 500- to 1000-mL separatory funnel. Allow the layers to separate.

10.3.1.3 Drain the aqueous layer back into the beaker.

10.3.1.4 The formation of emulsions can be expected in any solvent extraction procedure. If an emulsion forms, the analyst must take steps to break the emulsion before proceeding. Mechanical means of breaking the emulsion include the use of a glass stirring rod, filtration through glass wool, and other techniques. For emulsions that resist these techniques, centrifugation may be required.

If centrifugation is employed to break the emulsion, drain the organic layer into a centrifuge tube, cap the tube, and centrifuge for 2 to 3 minutes or until the phases separate. If the emulsion cannot be completely broken, collect as much as the organic phase as possible, and measure and record the volume of the organic phase collected.

If all efforts to break the emulsion fail, including centrifugation, and none of the organic phase can be collected, proceed with the dilute aliquot (Section 10.1.4.2). However, use of the dilute aliquot will sacrifice the sensitivity of the method, and may not be appropriate in all cases.

10.3.1.5 Drain the organic layer into a Kuderna-Danish (K-D) apparatus equipped with a 10-mL concentrator tube. Label the K-D apparatus. It may be necessary to pour the organic layer through a funnel containing anhydrous sodium sulfate to remove any traces of water from the extract.

10.3.1.6 Repeat the extraction (Section 10.3.1 through 10.3.5) two more times using another 200-mL of hexane for each extraction, combining the extracts in the K-D apparatus.

10.3.1.7 Repeat the derivatization and extraction for the remaining sample and QC aliquots.

10.3.1.8 Proceed with concentration of the extract, as described in Section 10.4.

10.3.2 Separatory funnel extraction of either final effluents or in-process wastewaters

10.3.2.1 Transfer the derivatized sample or QC aliquot to a 2-L separatory funnel.

10.3.2.2 Add 200 mL (±20 mL) of hexane to the separatory funnel. Cap the funnel and extract the sample by shaking the funnel for 2 to 3 minutes with periodic venting.

10.3.2.3 Allow the organic layer to separate from the water phase for a minimum of 10 minutes.

10.3.2.4 Drain the lower aqueous layer into the beaker used for derivatization (Section 10.2), or into a second clean 2-L separatory funnel. Transfer the solvent to a 1000-mL K-D flask. It may be necessary to pour the organic layer through a funnel containing anhydrous sodium sulfate to remove any traces of water from the extract.

10.3.2.5 The formation of emulsions can be expected in any solvent extraction procedure. If an emulsion forms, the analyst must take steps to break the emulsion before proceeding. Mechanical means of breaking the emulsion

include the use of a glass stirring rod, filtration through glass wool, and other techniques. For emulsions that resist these techniques, centrifugation may be required.

If centrifugation is employed to break the emulsion, drain the organic layer into a centrifuge tube, cap the tube, and centrifuge for 2 to 3 minutes or until the phases separate. If the emulsion cannot be completely broken, collect as much as the organic phase as possible, and measure and record the volume of the organic phase collected.

If all efforts to break the emulsion, including centrifugation, fail and none of the organic phase can be collected, proceed with the dilute aliquot (Section 10.1.4.2). However, use of the dilute aliquot will sacrifice the sensitivity of the method, and may not be appropriate in all cases.

10.3.2.6 If drained into a beaker, transfer the aqueous layer to the 2-L separatory funnel (Section 10.3.2.1). Perform a second extraction using another 200 mL of fresh solvent.

10.3.2.7 Transfer the extract to the 1000-mL K-D flask in Section 10.3.2.4.

10.3.2.8 Perform a third extraction in the same fashion as above.

10.3.2.9 Proceed with concentration of the extract, as described in Section 10.4.

10.4 Macro concentration: Concentrate the extracts in separate 1000-mL K-D flasks equipped with 10-mL concentrator tubes. Add 1 to 2 clean boiling chips to the flask and attach a three-ball macro-Snyder column. Prewet the column by adding approximately 1 mL of hexane through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lowers joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation.

10.5 Micro-concentration: Final concentration of the extracts may be accomplished using either a micro-Snyder column or nitrogen evaporation. If using a micro-Snyder column, add a clean boiling chip and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the column by adding approximately 0.5 mL hexane through the top. Place the apparatus in the hot water bath. Adjust the vertical position and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the liquid reaches an apparent volume of approximately 0.2 mL, remove the apparatus from the water bath and allow to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with approximately 0.2 mL of hexane. If using nitrogen evaporation, transfer the concentrator tube to a nitrogen evaporation device and direct a gentle stream of clean dry nitrogen into the concentrator. Rinse the sides of the concentrator tube with small volumes of hexane, and concentrate the extract to a final volume of 0.2 mL.

10.6 Quantitatively transfer the concentrated extract to a clean screw-cap vial using hexane to rinse the concentrator tube. Adjust the final volume to 0.5 mL. Seal the vial with a PTFE-lined lid, and mark the level on the vial. Label with the sample number and store in the dark at −20 to −10°C until ready for analysis.

11. GCMS ANALYSIS

11.1 Establish the following operating conditions:

Carrier gas flow:	Helium 30 cm/sec at 50°C
Injector temperature:	300°C
Initial temperature:	50°C
Temperature program:	8°C/min to 270°C
Final hold:	Until after 2,6-dichlorosyringaldehyde elutes

Optimize GC conditions for analyte separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, IPR and OPR aliquots, and samples.

11.2 Bring the concentrated extract (Section 10.6) or standard (Sections 6.13 and 6.14) to room temperature and verify that any precipitate has redissolved. Verify the level on the extract (Sections 6.13, 6.14, and 10.6) and bring to the mark with solvent if required.

11.3 Add 5 μL of the DFB instrument internal standard solution (Section 6.10) to the 0.5-mL extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. Mix thoroughly. (If the extract cannot be concentrated to 0.5 mL, add 1 μL of the DFB solution per 0.1 mL of extract).

11.4 Inject a 1 μL volume of the standard solution or extract using on-column or splitless injection. For 0.5 mL extracts, this 1 μL injection volume will contain 50 ng of the DFB internal standard. If an injection volume other than 1 μL is used, that volume must contain 50 ng of DFB.

Start the GC column temperature ramp upon injection. Start MS data collection after the solvent peak elutes. Stop data collection after the 2,6-dichlorosyringaldehyde peak elutes. Return the column to the initial temperature for analysis of the next sample.

12. SYSTEM AND LABORATORY PERFORMANCE

12.1 At the beginning of each 8-hour shift during which analyses are performed, analytical system performance is verified for all compounds. Analysis of DFTPP and the nominal 50 μg/mL OPR (Section 10.1.5) is used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples and blanks be analyzed.

12.2 DFTPP spectrum validity: Inject 1 μL of the DFTPP solution (Section 6.11) either separately or within a few seconds of injection of the OPR standard (Section 12.3) analyzed at the beginning of each shift. The criteria in Table 3 shall be met.

12.3 Calibration verification and ongoing precision and recovery: Analyze the extract of the OPR (Section 10.1.5) at the beginning of each 8-hour shift and prior to analysis of samples from the same batch. This aliquot serves as both the calibration verification and the ongoing precision and recovery test. Alternatively, a separate calibration verification may be performed using an aliquot of the midpoint calibration standard from Section 6.13 (with a nominal concentration of 50 μg/mL). This alternative may be used when samples extracted together with an OPR aliquot are not analyzed within the same 8-hour analysis shift.

12.3.1 Retention times: The absolute retention time of 2,2′-difluorobiphenyl shall be within the range of 765 to 885 seconds and the relative retention times of all pollutants and labeled compounds shall fall within the limits given in Table 2.

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

12.3.3 Multiple peaks: Each compound injected shall give a single, distinct GC peak.

12.3.4 Compute the concentration of each pollutant (Table 1) by isotope dilution (Section 7.4) for those compounds that have labeled analogs. Compute the concentration of each pollutant that has no labeled analog by the internal standard method (Section 7.5), using the 3,4,5-trichlorophenol (SMIS) as the internal standard. Compute the concentration of the labeled compounds and the SMIS by the internal standard method, using the 2,2′-difluorobiphenyl as the internal standard.

12.3.4.1 For each compound, compare the concentration with the limits for ongoing precision and recovery in Table 5. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, system performance is unacceptable for that compound. In this event, there may be a problem with the GCMS or with the derivatization/extraction/concentration systems.

12.3.4.2 GCMS system: If the reason for the failure of the OPR test (Section 12.3.4.1) is suspected to be the GCMS, prepare a fresh nominal 50 μg/mL calibration standard and test the GCMS again. If the problem persists, recalibrate the GCMS. However, if the performance of the GCMS system is verified, the derivatization/extraction/concentration steps are out of control. In this event, rederivatize the affected portion of the sample batch (see the note in Section 12.3.4.1) and repeat the performance tests (Sections 12.1 through 12.3).

12.4 Add results which pass the specifications in Section 12.3.4.1 to initial and previous ongoing data for each compound. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each pollutant and labeled compound in each matrix type (reagent water, C-stage filtrate, E-stage filtrate, final effluent, etc.) by calculating the average percent recovery (R) and the standard deviation of percent recovery (sr). Express the accuracy as a recovery interval from R − 2sr to R + 2sr. For example, if R = 95% and sr = 5%, the accuracy is 85 to 105%.

13. QUALITATIVE DETERMINATION

Identification is accomplished by comparison of data from analysis of a sample or blank with data stored in the mass spectral libraries. Identification of a compound is confirmed when the following criteria are met:

13.1 The signals for m/z 43 (to indicate the presence of the acetyl derivative) and all characteristic m/z's stored in the spectral library (Section 7.2.4) shall be present and shall maximize within the same two consecutive scans.

13.2 Excluding m/z's in the 35 to 45 range, either (1) the background corrected EICP areas, or (2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum shall agree within a factor of two (0.5 to 2 times) for all m/z's stored in the library.

13.3 The relative retention time shall be within the window specified in Table 2.

13.4 The m/z's present in the mass spectrum from the component in the sample that are not present in the reference mass spectrum shall be accounted for by contaminant or background ions. If the mass spectrum is contaminated, or if identification is ambiguous, an experienced spectrometrist (Section 1.4) is to determine the presence or absence of the compound.

14. QUANTITATIVE DETERMINATION

14.1 Isotope dilution: By adding a known amount of a labeled compound to every sample prior to derivatization and extraction, correction for recovery of the pollutant can be made because the pollutant and its labeled analog exhibit the same effects upon derivatization, extraction, concentration, and gas chromatography. Relative response (RR) values for sample mixtures are used in conjunction with calibration curves described in Section 7.4 to determine concentrations directly, so long as labeled compound spiking levels are constant. For the phenol example given in Figure 1 (Section 7.4.1), RR would be equal to 1.114. For this RR value, the phenol calibration curve given in Figure 1 indicates a concentration of 27 μg/mL in the sample extract (C_{ex}). Compute the concentration in the extract using the response factor determined from calibration data (Section 7.4) and the following equation:

$$C_{ex}(\mu g/mL) = (A_n \times C_l)(A_l \times RR)$$

Where:
C_{ex} = *concentration of the compound in the extract*
A_n = *area of the characteristic mass for the pollutant compound with a labeled analog*
C_l = *concentration of the labeled compound in the extract*
A_l = *area of the characteristic mass for the labeled compound*
RR = *response ratio from the initial calibration*

The concentration in extract (C_{ex}) is used for the evaluation of method and laboratory performance, in the form of IPR (Section 8.2) and OPR (Section 12.3).

14.2 Internal standard: Compute the concentration in the extract using the response factor determined from calibration data (Section 7.5) and the following equation:

$$C_{ex}(\mu g/mL) = (A_s \times C_{is})(A_{is} \times RF)$$

Where:
C_{ex} = *concentration of the compound in the extract*
A_s = *area of the characteristic mass for the pollutant compound without a labeled analog*
C_{is} = *concentration of the internal standard in the extract (see note below)*
A_{is} = *area of the characteristic mass for the internalstanard*
RF = *response factor from the initial calibration*

NOTE: When this equation is used to compute the extract concentrations of pollutant compounds without labeled analogs, use the area (A_{is}) and concentration (C_{is}) of 3,4,5-trichlorophenol (SMIS) as the internal standard.

The concentration in extract (C_{ex}) is used for the evaluation of method and laboratory performance, in the form of IPR (Section 8.2) and OPR (Section 12.3). Note that separate calibration curves will be required for samples with and without the addition of ascorbic acid, and also for both extraction procedures (stir-bar and separatory funnel) where applicable.

NOTE: Separate calibration curves will be required for samples with and without the addition of ascorbic acid, and also for both extraction procedures (stir-bar and separatory funnel) where applicable.

14.3 Compute the concentration of the labeled compounds and the SMIS using the equation in Section 14.2, but using the area and concentration of the 2,2'-difluorobiphenyl as the internal standard, and the area of the labeled compound or SMIS as A_s.

14.4 Compute the concentration of each pollutant compound in the sample using the following equation.

$$C_s(\mu g/L) = \frac{(C_{ex} \times V_{ex})}{V_o}$$

where:
C_s = *Concentration of the pollutant in the sample*
C_{ex} = *Concentration of the pollutant in the extract*
V_{ex} = *Volume of the concentrated extract (typically 0.5 mL)*
V_o = *Volume of the original sample in liters*

14.5 Compute the recovery of each labeled compound and the SMIS as the ratio of concentration (or amount) found to the concentration (or amount) spiked, using the following equation.

$$Percent\ recovery = \frac{Concentration\ found}{Concentration\ spiked} \times 100$$

14.6 If the EICP area at the quantitation m/z for any compound exceeds the calibration range of the system, three approaches are used to obtain results within the calibration range.

14.6.1 If the recovery of all the labeled compounds in the original sample aliquot meet the recovery limits in Table 5, then the extract of the sample may be diluted by a maximum of a factor of 10, and the diluted extract reanalyzed. The concentration of the DFB internal standard must be maintained at 50 μg/mL (Section 11.4) in the diluted extract. This may be accomplished by diluting the original extract with hexane that contains DFB at a concentration of 50 μg/mL.

14.6.2 If the recovery of any labeled compound is outside the limits in Table 5, or if a tenfold dilution of the extract will not bring the compound within the calibration range, then extract and analyze a dilute aliquot of the sample (Section 10). Dilute 100 mL, 10 mL, or an appropriate volume of sample to 1000 mL with reagent water and extract per Section 10.

14.6.3 If the sample holding time has been exceeded, then the original sample extract is diluted by successive factors of 10, the DFB internal standard is added to give a concentration of 50 μg/mL in the diluted extract, and the diluted extract is analyzed. Quantitation of all analytes is performed using the DFB internal standard.

14.7 Results are reported for all pollutants, labeled compounds, and the sample matrix internal standard in standards, blanks, and samples, in units of μg/L.

14.7.1 Results for samples which have been diluted are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 14.6.)

14.7.2 For compounds having a labeled analog, results are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 14.6) and the labeled compound recovery is within the normal range for the method (Section 15.4).

15. ANALYSIS OF COMPLEX SAMPLES

15.1 Some samples may contain high levels (> 1000 μg/L) of the compounds of interest, interfering compounds, and/or other phenolic materials. Some samples will not concentrate to 0.5 mL (Section 10.5); others will overload the GC column and/or mass spectrometer; others may contain amounts of phenols that may exceed the capacity of the derivatizing agent.

15.2 Analyze the dilute aliquot (Section 10.1.4) when the sample will not concentrate to 0.5 mL. If a dilute aliquot was not extracted, and the sample holding time (Section 9.4) has not been exceeded, dilute an aliquot of sample with reagent water, and derivatize and extract it (Section 10.1.4). Otherwise, dilute the extract (Section 14.6.3) and quantitate it by the internal standard method (Section 14.2).

15.3 Recovery of internal standard: The EICP area of the internal standard should be within a factor of two of the area in the OPR or CALVER standard (Section 12.3). If the absolute areas of the labeled compounds and the SMIS are within a factor of two of the respective areas in the OPR or CALVER standard, and the DFB internal standard area is less than one-half of its respective area, then internal standard loss in the extract has occurred. In this case, analyze the extract from the dilute aliquot (Section 10.1.4).

15.4 Recovery of labeled compounds: Labeled compound recovery specifications have been developed for samples with and without the addition of ascorbic acid. Compare the recovery of each labeled compound to the appropriate limits in Table 5.

15.4.1 If the labeled compound recovery is outside the limits given in Table 5, the extract from the dilute aliquot (Section 10) is analyzed as in Section 14.6.

15.4.2 If the recoveries of all labeled compounds and the internal standard are low (per the criteria above), then a loss in instrument sensitivity is the most likely cause. In this case, the 50 µg/mL OPR standard (Section 12.3) shall be analyzed and calibration verified (Section 12.3.4.1). If a loss in sensitivity has occurred, the instrument shall be repaired, the performance specifications in Section 12 shall be met, and the extract reanalyzed.

15.4.3 If a loss in instrument sensitivity has not occurred, the method does not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

16. METHOD PERFORMANCE

16.1 Single laboratory performance for this method is detailed in References 1, 2, and 11. Acceptance criteria were established from multiple laboratory use of the draft method.

16.2 A chromatogram of the ongoing precision and recovery standard (Section 6.14) is shown in Figure 4.

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Table 1. Chlorophenolic Compounds Determined by GCMS using Isotope Dilution and Internal Standard Techniques

	Pollutant			Labeled Compound	
Compound	**CAS Registry**	**EPA-EGD**	**Analog**	**CAS Registry**	**EPA-EGD**
4-chlorophenol	106-48-9	1001			
2,4-dichlorophenol	120-83-2	1002	d_3	93951-74-7	1102
2,6-dichlorophenol	87-65-0	1003			
2,4,5-trichlorophenol	95-95-4	1004			
2,4,6-trichlorophenol	88-06-2	1005			
2,3,4,6-tetrachlorophenol	58-90-2	1006			
pentachlorophenol	87-86-5	1007	$^{13}C_6$	85380-74-1	1107
4-chloroguaiacol	16766-30-6	1008	$^{13}C_6$	136955-39-0	1108
3,4-dichloroguaiacol	77102-94-4	1009			
4,5-dichloroguaiacol	2460-49-3	1010			
4,6-dichloroguaiacol	16766-31-7	1011			
3,4,5-trichloroguaiacol	57057-83-7	1012			
3,4,6-trichloroguaiacol	60712-44-9	1013			
4,5,6-trichloroguaiacol	2668-24-8	1014	$^{13}C_6$	136955-40-3	1114
tetrachloroguaiacol	2539-17-5	1015	$^{13}C_6$	136955-41-4	1115
4-chlorocatechol	2138-22-9	1016			
3,4-dichlorocatechol	3978-67-4	1017			
3,6-dichlorocatechol	3938-16-7	1018			
4,5-dichlorocatechol	3428-24-8	1019	$^{13}C_6$	136955-42-5	1119
3,4,5-trichlorocatechol	56961-20-7	1020			
3,4,6-trichlorocatechol	32139-72-3	1021			
tetrachlorocatechol	1198-55-6	1022	$^{13}C_6$	136955-43-6	1122
5-chlorovanillin	19463-48-0	1023	$^{13}C_6$	136955-44-7	1123
6-chlorovanillin	18268-76-3	1024			
5,6-dichlorovanillin	18268-69-4	1025			
2-chlorosyringaldehyde	76341-69-0	1026			
2,6-dichlorosyringaldehyde	76330-06-8	1027			
trichlorosyringol	2539-26-6	1028			
Sample matrix internal standard (SMIS)					
3,4,5-trichlorophenol	609-19-8	184			
Instrument internal standard (IIS)					
2,2'-difluorobiphenyl	388-82-9				164

Table 2. Gas Chromatography and Method Detection Limits for Chlorophenolics

EGD No.[1]	Compound	Retention Time Mean (sec)[2]	EGD Ref No.	RRT Window[3]	Minimum Level[4] (µg/L)	MDL[5] (µg/L)
1001	4-chlorophenol	691	184	0.651–0.681	1.25	1.11
1003	2,6-dichlorophenol	796	184	0.757–0.779	2.5	1.39
1102	2,4-dichlorophenol-d_3	818	164	0.986–0.998		
1202	2,4-dichlorophenol	819	1102	0.997–1.006	2.5	0.15
164	2,2'-difluorobiphenyl (I.S.)	825	164	1.000		
1108	4-chloroguaiacol-$^{13}C_6$	900	164	1.077–1.103		
1208	4-chloroguaiacol	900	1108	0.998–1.002	1.25	0.09
1005	2,4,6-trichlorophenol	920	184	0.879–0.895	2.5	0.71
1004	2,4,5-trichlorophenol	979	184	0.936–0.952	2.5	0.57
1016	4-chlorocatechol	1004	184	0.961–0.975	1.25	0.59
1011	4,6-dichloroguaiacol	1021	184	0.979–0.991	2.5	0.45
1009	3,4-dichloroguaiacol	1029	184	0.986–0.998	2.5	0.52
184	3,4,5-trichlorophenol (I.S.)	1037	164	1.242–1.272		
1010	4,5-dichloroguaiacol	1071	184	1.026–1.040	2.5	0.52
1018	3,6-dichlorocatechol	1084	184	1.037–1.053	2.5	0.57
1006	2,3,4,6-tetrachlorophenol	1103	184	1.050–1.078	2.5	0.38
1123	5-chlorovanillin-$^{13}C_6$	1111	164	1.327–1.367		
1223	5-chlorovanillin	1111	1123	0.998–1.001	2.5	1.01
1013	3,4,6-trichloroguaiacol	1118	184	1.066–1.090	2.5	0.46
1024	6-chlorovanillin	1122	184	1.070–1.094	2.5	0.94
1017	3,4-dichlorocatechol	1136	184	1.083–1.105	2.5	0.60
1119	4,5-dichlorocatechol-$^{13}C_6$	1158	164	1.384–1.424		
1219	4,5-dichlorocatechol	1158	1119	0.998–1.001	2.5	0.24
1012	3,4,5-trichloroguaiacol	1177	184	1.120–1.160	2.5	0.49
1114	4,5,6-trichloroguaiacol-$^{13}C_6$	1208	164	1.444–1.484		
1214	4,5,6-trichloroguaiacol	1208	1114	0.998–1.002	2.5	0.25
1021	3,4,6-trichlorocatechol	1213	184	1.155–1.185	5.0	0.44
1025	5,6-dichlorovanillin	1246	184	1.182–1.222	5.0	0.80
1026	2-chlorosyringaldehyde	1255	184	1.190–1.230	2.5	0.87
1107	pentachlorophenol-$^{13}C_6$	1267	164	1.511–1.561		
1207	pentachlorophenol	1268	1107	0.998–1.002	5.0	0.28
1020	3,4,5-trichlorocatechol	1268	184	1.208–1.238	5.0	0.53
1115	tetrachloroguaiacol-$^{13}C_6$	1289	164	1.537–1.587		
1215	tetrachloroguaiacol	1290	1115	0.998–1.002	5.0	0.23
1028	trichlorosyringol	1301	184	1.240–1.270	2.5	0.64

Table 2. Gas Chromatography and Method Detection Limits for Chlorophenolics (continued)

EGD No.[1]	Compound	Retention Time Mean (sec)[2]	EGD Ref No.	RRT Window[3]	Minimum Level[4] (µg/L)	MDL[5] (µg/L)
1122	tetrachlorocatechol-$^{13}C_6$	1365	164	1.630–1.690		
1222	tetrachlorocatechol	1365	1122	0.998–1.002	5.0	0.76
1027	2,6-dichlorosyringaldehyde	1378	184	1.309–1.349	5.0	1.13

1. Four digit numbers beginning with 10 indicate a pollutant quantified by the internal standard method; four digit numbers beginning with 11 indicate a labeled compound quantified by the internal standard method; four digit numbers beginning with 12 indicate a pollutant quantified by isotope dilution.
2. The retention times in this column are based on data from a single laboratory (reference 12), utilizing the GC conditions in Section 11.
3. Relative retention time windows are estimated from EPA Method 1625.
4. The Minimum Level is defined as the concentration in a sample equivalent to the concentration of the lowest calibration standard analyzed in the initial calibration, assuming that all the method-specified sample volumes are utilized. It is the lowest level at which the analytical system will give recognizable mass spectra (background corrected) and acceptable calibration points.
5. 40 *CFR* Part 136, Appendix B; from reference 2.

Table 3. DFTPP Mass Intensity Specifications[1]

Mass	*Intensity Required*
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% 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

1. Reference 7

Table 4. Characteristic m/z's of Chlorophenolic Compounds

Compound	*Primary m/z*
2,4-dichlorophenol	162
2,4-dichlorophenol-d_3	167
2,6-dichlorophenol	162
2,4,5-trichlorophenol	196
2,4,6-trichlorophenol	196
2,3,4,6-tetrachlorophenol	232
pentachlorophenol	266
pentachlorophenol-$^{13}C_6$	272
4-chloroguaiacol	158
4-chloroguaiacol-$^{13}C_6$	164
3,4-dichloroguaiacol	192
4,5-dichloroguaiacol	192
4,6-dichloroguaiacol	192
3,4,5-trichloroguaiacol	226
3,4,6-trichloroguaiacol	226
4,5,6-trichloroguaiacol	226
4,5,6-trichloroguaiacol-$^{13}C_6$	234
tetrachloroguaiacol	262
tetrachloroguaiacol-$^{13}C_6$	268
4-chlorocatechol	144
3,4-dichlorocatechol	178
3,6-dichlorocatechol	178
4,5-dichlorocatechol	178
4,5-dichlorocatechol-$^{13}C_6$	184
3,4,5-trichlorocatechol	212
3,4,6-trichlorocatechol	212
tetrachlorocatechol	248
tetrachlorocatechol-$^{13}C_6$	254
5-chlorovanillin	186
5-chlorovanillin-$^{13}C_6$	192
6-chlorovanillin	186
5,6-dichlorovanillin	220
2-chlorosyringaldehyde	216
2,6-dichlorosyringaldehyde	250
trichlorosyringol	256
Sample Matrix Internal Standard (SMIS)	
3,4,5-trichlorophenol	196
Instrument Internal Standard (IIS)	
2,2'-difluorobiphenyl	190

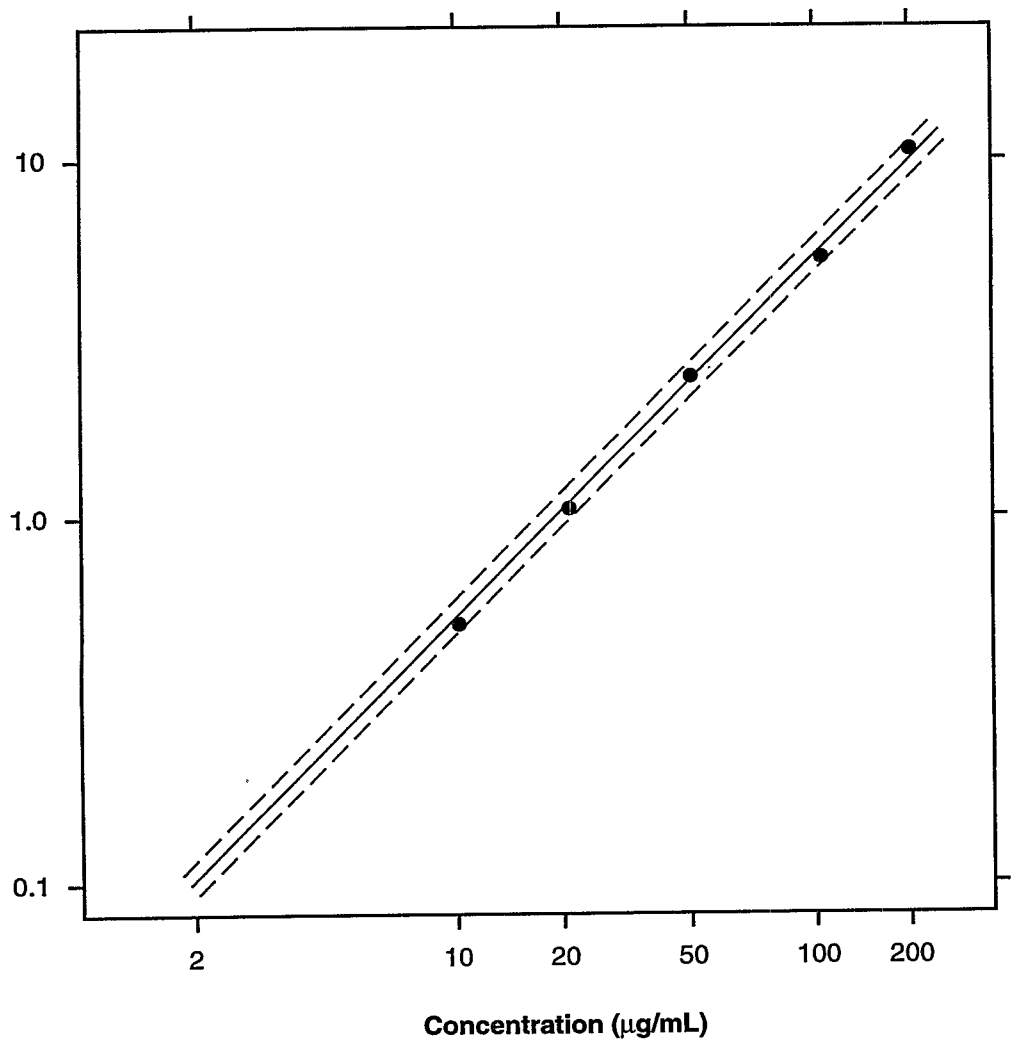
Table 5. Acceptance Criteria for Performance Tests[1]

EGD No.[2]	Compound	Test conc.[3] (µg/mL)	Initial precision and accuracy Sec. 8.2.3 (µg/mL)		Ongoing accuracy Sec. 12.3 (µg/mL)	Labeled compound and SMIS recovery Sec. 8.3 and 14.5	
			s	X		without ascorbic acid P (%)	with ascorbic acid P (%)
1001	4-chlorophenol	25	16	18–36	10–59		
1202	2,4-dichlorophenol	50	7	42–60	42–59		
1102	2,4-dichlorophenol-d_3	50	27	32–80	28–85	58–135	27–143
1003	2,6-dichlorophenol	50	10	33–74	29–85		
1004	2,4,5-trichlorophenol	50	7	39–70	41–64		
1005	2,4,6-trichlorophenol	50	10	36–71	36–73		
1006	2,3,4,6-tetrachlorophenol	50	7	40–66	41–66		
1207	pentachlorophenol	100	6	90–111	84–120		
1107	pentachlorophenol-$^{13}C_6$	100	21	58–169	61–157	8–143	27–167
1208	4-chloroguaiacol	25	5	22–30	22–30		
1108	4-chloroguaiacol-$^{13}C_6$	25	26	17–37	16–38	59–121	43–168
1009	3,4-dichloroguaiacol[4]	50	9	40–63	41–63		
1010	4,5-dichloroguaiacol	50	7	41–61	40–64		
1011	4,6-dichloroguaiacol	50	8	41–63	43–60		
1012	3,4,5-trichloroguaiacol	50	8	39–65	40–67		
1013	3,4,6-trichloroguaiacol	50	8	32–76	37–70		
1214	4,5,6-trichloroguaiacol	50	7	46–53	44–58		
1114	4,5,6-trichloroguaiacol-$^{13}C_6$	50	24	33–73	37–70	48–131	51–139
1215	tetrachloroguaiacol	100	7	84–115	81–126		
1115	tetrachloroguaiacol-$^{13}C_6$	100	22	57–173	65–161	35–120	27–161
1016	4-chlorocatechol	25	12	19–35	20–31		
1017	3,4-dichlorocatechol	50	12	33–77	39–67		
1018	3,6-dichlorocatechol	50	8	39–68	42–63		
1219	4,5-dichlorocatechol	50	4	42–59	43–61		
1119	4,5-dichlorocatechol-$^{13}C_6$	50	39	34–72	33–71	33–129	0–190
1020	3,4,5-trichlorocatechol	100	17	60–166	72–128		
1021	3,4,6-trichlorocatechol[4]	100	17	74–138	64–149		
1222	tetrachlorocatechol	100	29	46–234	81–132		
1122	tetrachlorocatechol-$^{13}C_6$	100	39	48–227	63–152	14–118	0–184
1223	5-chlorovanillin	50	10	47–104	42–59		
1123	5-chlorovanillin-$^{13}C_6$	50	42	34–80	35–72	51–126	32–254
1024	6-chlorovanillin	50	11	41–64	40–63		
1025	5,6-dichlorovanillin	100	9	67–146	77–140		

Table 5. Acceptance Criteria for Performance Tests (continued)[1]

EGD No.[2]	Compound	Test conc.[3] (µg/mL)	Initial precision and accuracy Sec. 8.2.3 (µg/mL)		Ongoing accuracy Sec. 12.3 (µg/mL)	Labeled compound and SMIS recovery Sec. 8.3 and 14.5	
			s	X		without ascorbic acid P (%)	with ascorbic acid P (%)
1026	2-chlorosyringaldehyde	50	14	38–65	36–78		
1027	2,6-dichlorosyringaldehyde	100	14	82–129	60–183		
1028	trichlorosyringol	50	9	38–68	33–87		
Sample Matrix Internal Standard							
184	3,4,5-trichlorophenol	100	47	62–185	68–144	56–116	24–167

1. Specifications derived from multi-laboratory testing of draft method.
2. Four-digit numbers beginning with 10 indicate a pollutant quantified by the internal standard method; four-digit numbers beginning with 11 indicate a labeled compound quantified by the internal standard method; four-digit numbers beginning with 12 indicate a pollutant quantified by isotope dilution.
3. Test concentrations and IPR and OPR results are all calculated and evaluated as concentrations in extract, in units of µg/mL.
4. Specification derived from isomer.



The dotted lines enclose a ±10% error window.

52-020-21A

Figure 1. Relative Response Calibration Curve for Phenol

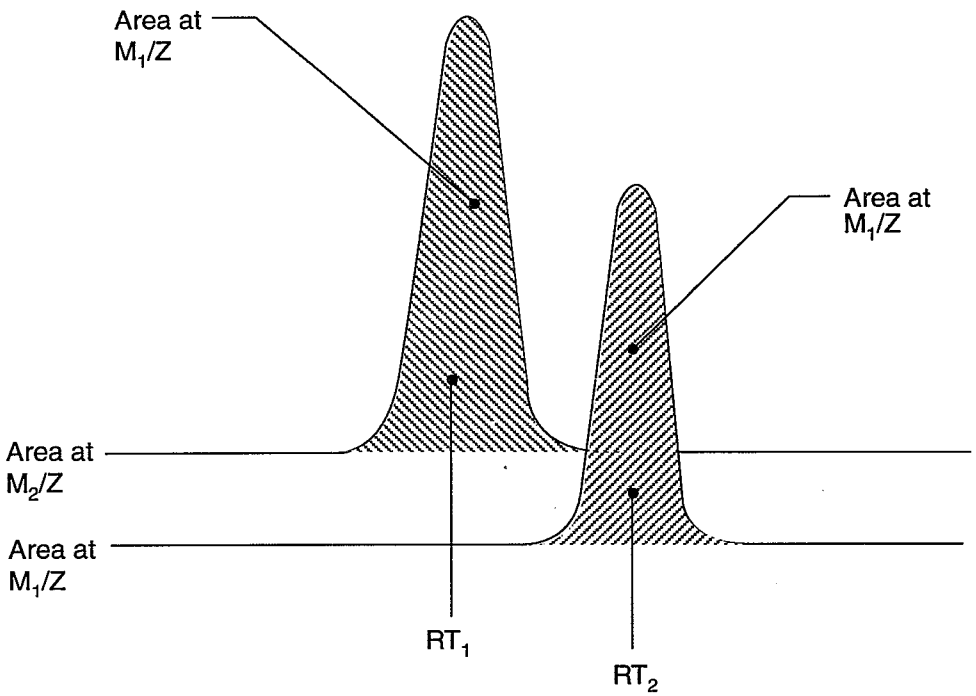


Figure 2. Extracted Ion-Current Profiles for Chromatographically Resolved Labeled (M_2/Z) and Unlabeld (M_1/Z) Pairs

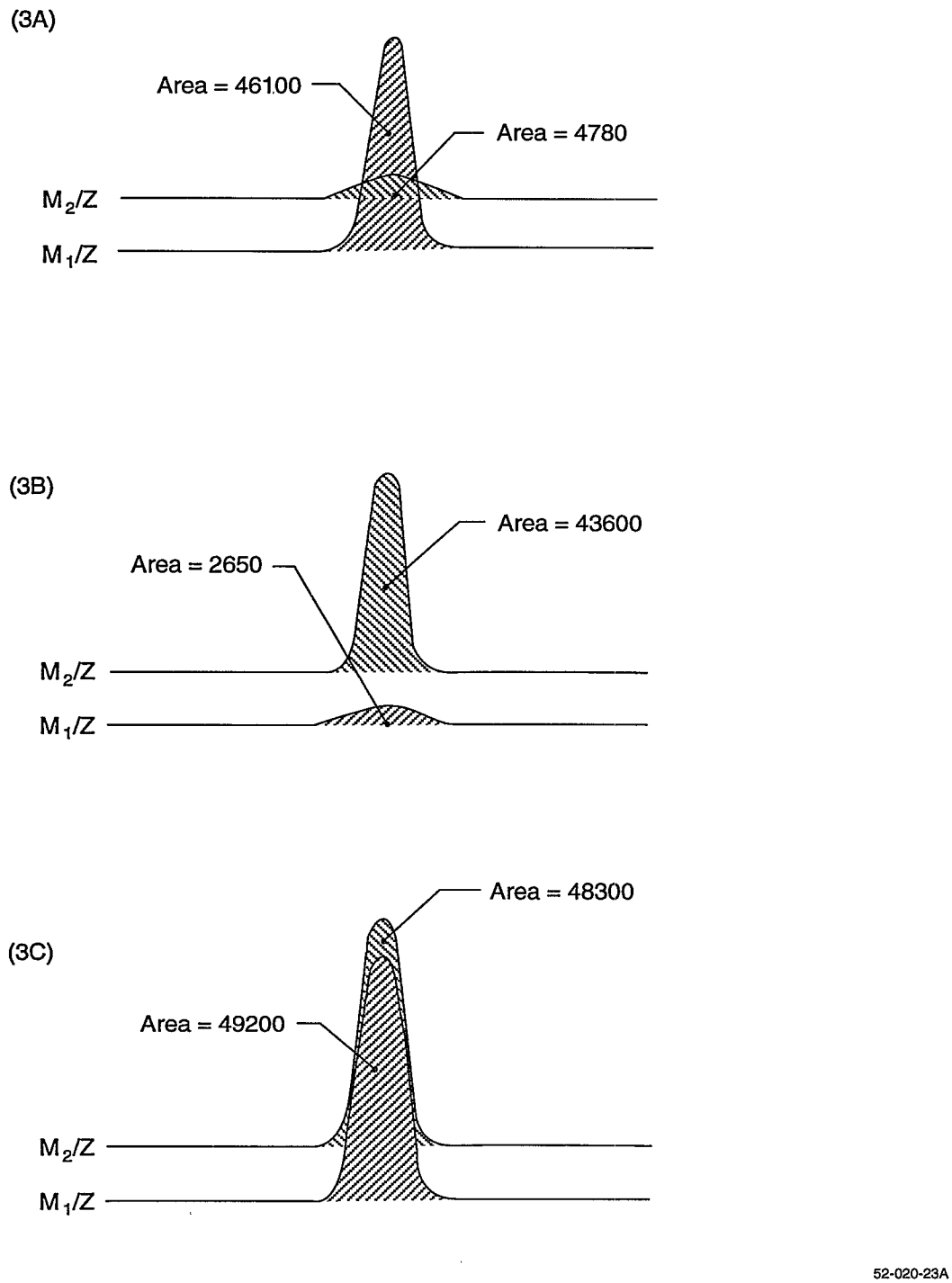


Figure 3. Extracted Ion-Current Profiles for (3A) Unlabeled Compound, (3B) Labeled Compound, and (3C) Equal Mixture of Unlabeled and Labeled Compounds

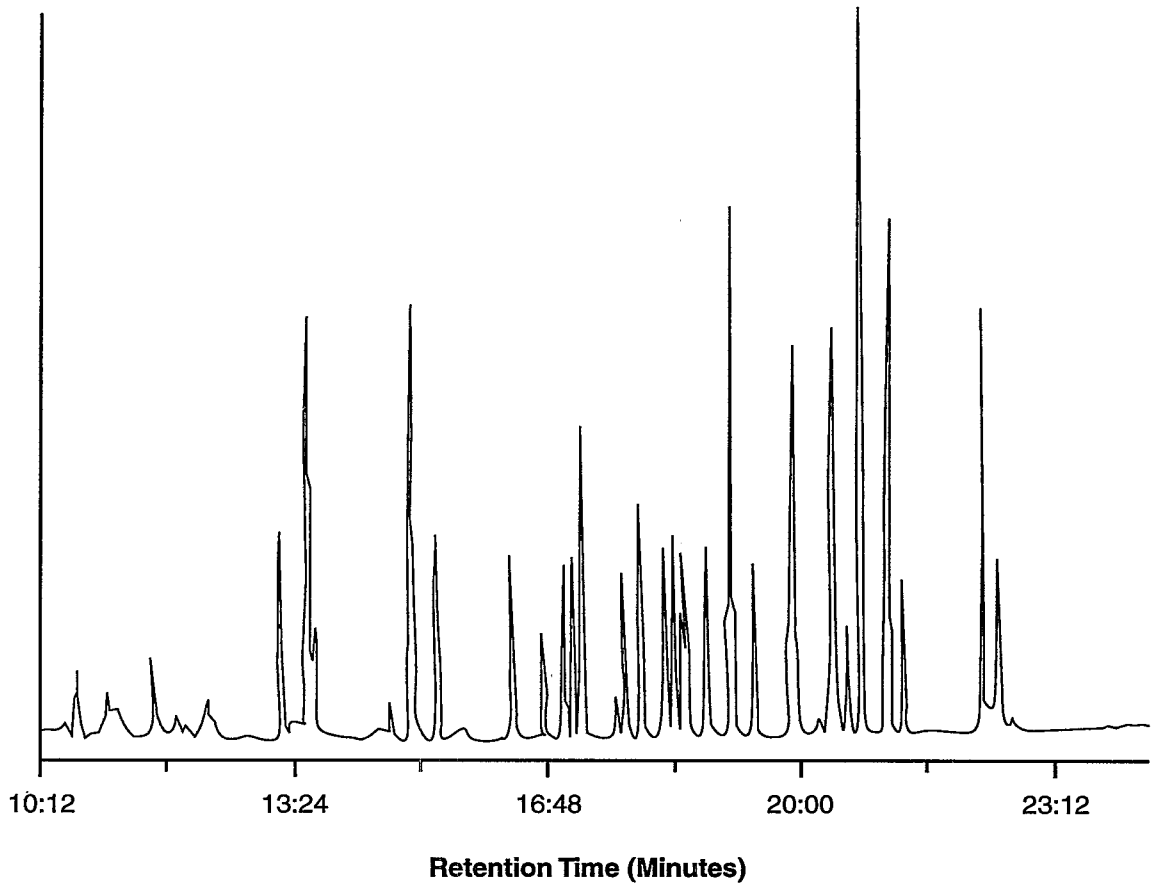


Figure 4. Chromatogram of Chlorophenolics

Method NCASI 253

Tentative Procedure for Color Measurement of Pulping Wastes and Their Receiving Waters—Spectrophotometric Method

June 1978

Addendum to NCASI Method 253: Quality Control

1. Scope and Application

1.1 NCASI 253 is an industry-developed method designed to determine the color of wastewater discharged by the Pulp and Paper Industry. Data collected with this method were used by the EPA to develop regulations for these discharges. As written by the industry, the method contains little quality control (QC). The QC requirements given below are to be used when compliance data are collected using this method.

1.2 This QC is consistent with the QC requirements specified in the methods in 40 *CFR* Part 136 Appendix A promulgated October 26, 1984 (49 *FR* 43234).

2. Calibration and Calibration Verification

2.1 Section 4 of NCASI 253 requires the analyst to "construct a calibration curve by plotting absorbance values against color units." This curve shall be assumed linear if the correlation coefficient is equal to or greater than 0.991. If the correlation coefficient is less than 0.991, repeat the calibration.

2.2 Calibration shall be verified after every tenth sample by analyzing a standard at the midpoint of the calibration curve. If the calculated recovery from the curve is not in the range of 90 to 110% of the true value, the instrument must be recalibrated and the samples analyzed prior to the failed calibration must be reanalyzed.

3. Initial Precision and Recovery (IPR)

3.1 Prepare a stock chloroplatinate standard and spike four aliquots of reagent water (pH 7.6) to produce a concentration of 100 color units/L.

3.2 Filter and analyze the four aliquots. The average color value must be in the range of 80 to 120% and the standard deviation must be less than 10%. If either of these criteria are not met, the analytical system is not in control. Correct the problem and repeat the test.

4. Ongoing Precision and Recovery (OPR)

4.1 With each batch of samples analyzed on the same 8-hour shift (to a maximum of ten samples), analyze a single ongoing precision and recovery standard (laboratory control sample) in the same manner as the IPR.

4.2 The OPR recovery must be in the range of 75 to 125%. If this criterion is not met, the analytical system is not in control. Correct the problem, and reanalyze the batch of samples with a new LCS.

Method NCASI 253

Tentative Procedure for Color Measurement of Pulping Wastes and Their Receiving Waters—Spectrophotometric Method

1. *INTRODUCTION*

The color of pulping waste or its receiving water is considered to be the color of the light transmitted by the waste solution after removing the suspended material, including the pseudocolloidal particles. It is recognized that the color characteristics of some wastes are affected by the light reflection from the suspended material in the wastes.

The term "color" is used herein to mean "true color"—that is, the color of the water from which the turbidity has been removed.

2. *GENERAL DISCUSSION*

a. Principle: Color is determined by spectrophotometric comparison of the sample with known concentrations of colored solutions. The platinum-cobalt method of measuring color is given as the standard method, the unit of color being that produced by 1 mg/L platinum, in the form of chloroplatinate ion. The ratio of cobalt to platinum may be varied to match the hue in special cases; the proportion given below is usually satisfactory to match the color of natural waters.

b. Interference: Even a slight turbidity causes the measured color to be noticeably higher than the true color; therefore, it is necessary to remove turbidity before true color can be measured by spectrophotometric comparison. The recommended method for removal of turbidity is filtration through a membrane filter having a median porosity of 0.8 microns.

The color value of a water sample is highly pH dependent, and invariably increases as the pH of the water is raised. For this reason it is necessary, when reporting a color value, to adjust the pH of all samples to 7.6 with 1 N HCl or 1 N NaOH solution.

c. Sampling: Samples for the color determination should be representative and must be taken in clean glassware. The color determination should be made within a reasonable period, as biologic changes occurring in storage may affect the color, and alter the pH value.

3. *APPARATUS*

a. Spectrophotometer, having absorption cells of the following length for minimum detectable color, a narrow (10 mμ or less) spectral band and an effective operating range from 400 to 700 mμ.

Absorption Cell Length, Centimeters	Minimum Detectable Color Units
30	1
20	5
10	8
5	10
1	25

b. pH meter, for determining the sample pH.

c. Filtration system, consisting of flask, vacuum source, filter holder, and 0.8 micron porosity membrane filters.

4. PREPARATION OF STANDARDS

If a reliable potassium chloroplatinate standard solution cannot be purchased from a laboratory supply house, it may be replaced by chloroplatinic acid, which the analyst can prepare from metallic platinum. Commercial chloroplatinic acid should not be used because it is very hygroscopic and therefore may vary in platinum content. Potassium chloroplatinate is not hygroscopic.

Dissolve 1.246 g potassium chloroplatinate, K_2PtCl_6 (equivalent to 0.500 g metallic platinum) and 1 g crystallized cobaltous chloride, $CoCl_2 \cdot 6H_2O$ (equivalent to about 0.25 g metallic cobalt) in distilled water with 100 mL concentrated HCl and dilute to 1 liter with distilled water. This stock standard has a color of 500 units.

If potassium chloroplatinate is not available, dissolve 0.500 g pure metallic platinum in aqua regia with the aid of heat; remove nitric acid by repeated evaporation with fresh portions of concentrated HCl. Dissolve this product together with 1 g crystallized cobaltous chloride as directed above.

Prepare standards having colors of 25, 50, 100, 150, 200, and 250 by diluting 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0 mL stock color standard with distilled water to 50 mL in stoppered volumetric flasks. Protect these standards against evaporation and contamination when not in use.

Transfer a suitable portion of each final solution to a 10 mm absorption cell from a "matched set" of cells and measure the absorbance at 465 mμ. As reference use distilled water for instrument calibration to zero absorbance.

Construct a calibration curve by plotting absorbance values against color units similar to the sample curve presented in Figure 1.

Appropriately lower color standards should be used for the calibration curve with longer absorption cells. For example, employing the 10 to 20 centimeter cells, use standards of 5, 10, 15, 20, 50, 75, and 100 units of color, and develop the curve similar to the appropriate cell length illustrated by the set of curves presented in Figure 2.

5. PROCEDURE

Preparation of sample: Select a 200 mL sample of waste or water, adjust pH to 7.6 with HCl or NaOH as indicated in Section 2. If the overall volume change is greater than one percent, discard the sample and start anew with stronger solutions of HCl or NaOH for the pH adjustment. In any event, the volume change in the final sample should be no more than one percent.

Take a 50 mL aliquot of the pH adjusted sample and filter through a 0.8 micron porosity membrane filter prerinsed with distilled water. Then transfer an appropriate portion of the filtered sample to a 10 mm absorption cell and measure its absorbance at 465 mμ, using distilled water for the blank.

If the sample contains very high concentrations of turbidity (200 to 1000 J.T.U.) successively smaller aliquots of the sample should be used per membrane filter. This possibility may require filtration of 2 or 3 aliquots to accumulate sufficient sample to fill the appropriate absorption cell. The guideline to follow in selection of aliquot volume to filter should be based on the visual appearance of a sudden rapid reduction in filtration rate through the filter. This phenomenon would indicate the beginning of filter plugging, and that possible loss of color would result on further filtration of the sample.

Filtration should be stopped immediately on this occurrence, and the filter replaced with a clean pre-rinsed filter. Pre-rinsing of the filter with distilled water is recommended to prevent any change in pH resulting from use of "acid washed" filters.

Calculate the color units in the sample by comparing the absorbance reading with a standard curve secured by carrying out the procedure indicated in Section 4.

Report the color results in whole numbers and record as follows:

Color Units	*Record to Nearest*
1–100	1
101–500	5

Related Data Tabulations

Table A1. Color Measurement Study Data—Unbleached Kraft Effluent

Instrument No.	Color Units—pH Value, 465 mμ					
	7.0	7.2	7.4	7.6	7.8	8.0
1	350	360	364	360	370	367
2	352	324	305	324	362	324
3	363	363	363	363	366	373
4	331	350	338	344	350	364
5	326	333	333	340	340	336
6	--	--	--	466	--	--
7	366	376	357	384	366	385
8	300	310	315	325	330	330
9	325	339	366	374	388	388
10	384	394	404	398	415	426
11	366	398	351	358	386	390
12	391	391	508	470	508	470
13	585	624	650	676	702	728
14	302	302	300	321	317	331
15	337	338	349	342	343	354
16	345	350	348	363	350	351
17	405	405	405	405	410	410
18	280	280	300	300	300	300
Avg.	359	366	373	384	388	389
Min.	280	280	300	300	300	300
Max.	585	624	650	676	702	728
Color Increase		7	14	20	29	30

Table A2. Color Measurement Study Data—Bleached Kraft Effluent

Instrument No.	Color Units—pH Value, 465 mμ					
	7.0	7.2	7.4	7.6	7.8	8.0
1	235	235	235	235	235	244
2	216	207	226	226	220	226
3	252	252	252	252	260	260
4	338	343	343	351	350	369
5	333	340	340	400	404	414
6	--	--	--	482	--	--
7	310	324	343	343	343	362
8	340	340	350	350	360	360
9	325	325	325	325	329	335
10	318	322	386	380	391	404
11	245	298	288	308	326	316
12	314	352	470	430	430	508
13	370	400	420	440	460	480
14	462	328	336	342	350	356
15	340	342	342	342	342	342
16	--	--	--	--	--	--
17	--	--	--	--	--	--
18	220	220	220	220	220	230
Avg.	307	308	325	339	334	347
Min.	216	207	220	220	220	226
Max.	462	400	470	440	460	508
Color Increase		1	18	22	27	40

Table A3. Color Measurement Study Data—Caustic Extraction Effluent

Instrument No.	Color Units—pH Value, 465 mμ					
	7.0	7.2	7.5	7.6	7.8	8.0
1	127	121	121	121	113	121
2	193	169	188	151	202	193
3	201	201	204	207	207	211
4	167	167	172	167	193	187
5	192	192	189	252	192	192
6	--	--	--	248	--	--
7	188	179	193	178	188	197
8	145	150	150	150	155	160
9	201	225	242	266	276	294
10	184	172	196	184	196	207
11	179	175	193	183	204	182
12	117	117	157	353	196	196
13	150	180	200	220	230	230
14	171	171	174	174	185	174
15	140	141	143	144	145	145
16	186	186	181	208	184	182
17	220	215	210	208	208	207
18	160	160	180	180	180	180
Avg.	171	171	182	199	191	191
Min.	117	117	121	121	113	121
Max.	220	225	242	353	276	294
Color Increase		0	11	25	20	20

Table A4. Color Measurement Study Data—Natural Receiving Water

Instrument No.	Color Units—pH Value, 465 mμ					
	7.0	7.2	7.4	7.6	7.8	8.0
1	49	46	45	51	46	47
2	113	85	61	89	108	103
3	117	117	121	121	124	124
4	66	66	75	81	84	66
5	--	--	--	--	--	--
6	--	--	--	--	--	--
7	75	75	75	94	85	85
8	100	110	110	120	120	120
9	73	73	73	83	83	83
10	69	80	104	97	115	115
11	53	67	74	70	70	92
12	39	39	39	39	39	63
13	105	105	110	110	115	120
14	100	100	107	93	81	88
15	98	98	103	103	103	104
16	97	100	101	103	103	103
17	120	120	120	120	122	123
18	70	70	70	70	70	70
Avg.	84	84	87	90	92	94
Min.	39	39	39	39	39	63
Max.	120	120	121	121	124	124
Color Increase		0	3	6	8	10

Table A5. Color Measurement Study—Unbleached Kraft Effluent Color Units

Instrument No.	Wavelength in Millimicrons							
	455	460	465	470	475	480	485	490
1	358	352	360	361	375	387	637	615
2	--	--	324	--	--	--	--	367
3	--	--	363	--	--	--	--	428
4	352	364	344	349	361	353	390	418
5	352	341	340	335	348	350	370	386
7	336	335	384	348	383	391	408	470
9	413	345	374	337	373	389	408	440
10	482	383	398	375	417	440	442	454
11	392	385	358	368	383	360	406	405
12	509	564	470	391	564	517	528	783
14	316	297	321	308	293	348	377	358
16	392	369	363	364	369	381	390	414
Min.	509	564	470	391	564	517	637	783
Max.	316	297	321	308	293	348	370	358
Avg.	390	373	366	353	386	391	435	461

Table A6. Color Measurement Study—Bleached Kraft Effluent Color Units

Instrument No.	Wavelength in Millimicrons							
	455	460	465	470	475	480	485	490
1	233	253	235	248	237	241	406	388
2	--	--	226	--	--	--	--	254
3	--	--	252	--	--	--	--	263
4	364	384	351	363	368	362	397	426
5	416	352	400	343	411	416	438	460
7	313	305	343	320	326	347	371	426
9	363	345	325	327	320	328	345	357
10	445	387	380	366	391	425	549	423
11	308	307	308	319	313	282	316	322
12	509	329	430	235	517	470	376	626
14	362	304	342	308	326	386	430	388
16	--	--	--	--	--	--	--	--
Max.	509	387	430	366	517	470	549	626
Min.	233	253	235	235	237	241	316	254
Avg.	368	373	299	314	356	362	403	397

Table A7. Color Measurement Study Data—Caustic Extraction Stage Color Units

Instrument No.	Wavelength in Millimicrons							
	455	460	465	470	475	480	485	490
1	121	135	121	128	112	110	179	170
2	--	--	151	--	--	--	--	205
3	--	--	207	--	--	--	--	193
4	189	208	167	197	170	169	181	189
5	261	201	252	195	258	263	275	288
7	180	184	178	188	173	185	173	205
9	295	210	266	192	261	271	288	303
10	235	200	184	170	195	211	198	194
11	228	210	183	208	195	188	194	184
12	430	235	353	117	376	423	411	548
14	198	179	174	168	158	191	230	219
16	213	204	208	194	205	211	220	223
Max.	430	235	353	208	376	423	411	548
Min.	121	135	121	117	112	110	173	170
Avg.	235	196	203	175	200	222	234	243

Table A8. Color Measurement Study Data—Receiving Water Color Units

Instrument No.	Wavelength in Millimicrons							
	455	460	465	470	475	480	485	490
1	52	52	51	40	47	46	71	64
2	--	--	89	--	--	--	--	97
3	--	--	121	--	--	--	--	88
4	80	86	81	80	62	63	72	73
5	--	--	--	--	--	--	--	--
7	90	80	94	94	81	100	92	110
9	99	65	83	54	78	81	88	88
10	136	129	97	121	104	102	106	100
11	84	87	70	66	82	78	85	78
12	39	47	39	15	19	19	12	15
14	102	110	93	110	84	87	94	79
16	110	116	103	105	106	110	104	101
Max.	136	119	121	121	104	102	106	110
Min.	39	47	39	15	19	19	12	15
Avg.	88	85	84	76	73	76	80	81

Table A9. Color Measurement Study Data—Non-Specific Wavelength Colorimetric Instruments Color Units

Instrument Number	*Chloroplatinate Standard*	*Unbleached Kraft*	*Bleached Kraft*	*Caustic Extract*	*Receiving Water*
6	246	466	482	248	--
8	200	325	350	150	120
13	225	676	440	220	110
15	196	342	342	144	103
17	245	405	--	208	120
18	200	300	220	180	70
Max.	246	676	482	248	120
Min.	196	300	220	144	70
Avg.	218	419	366	191	104

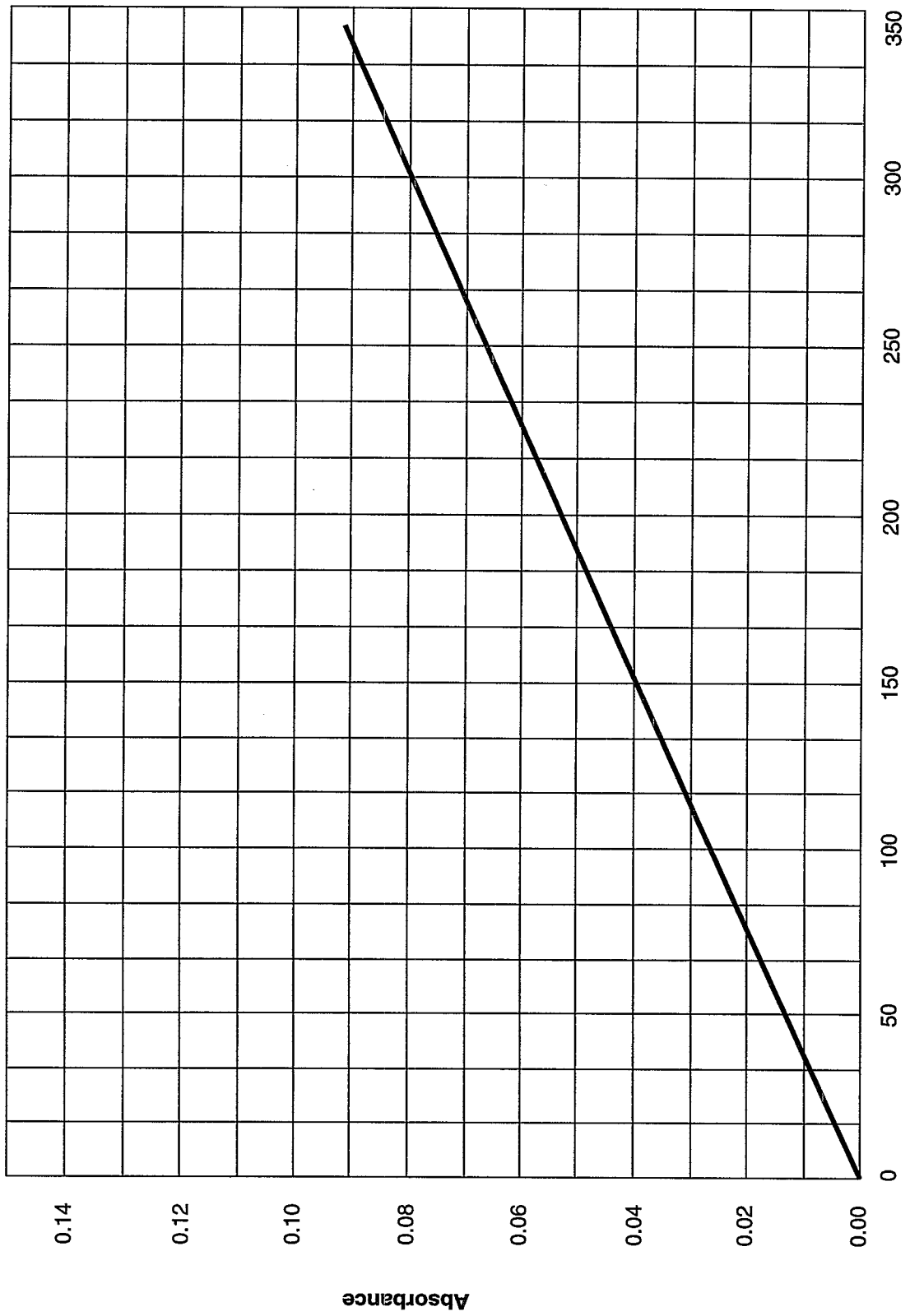


Figure 1. Typical Calibration Curve

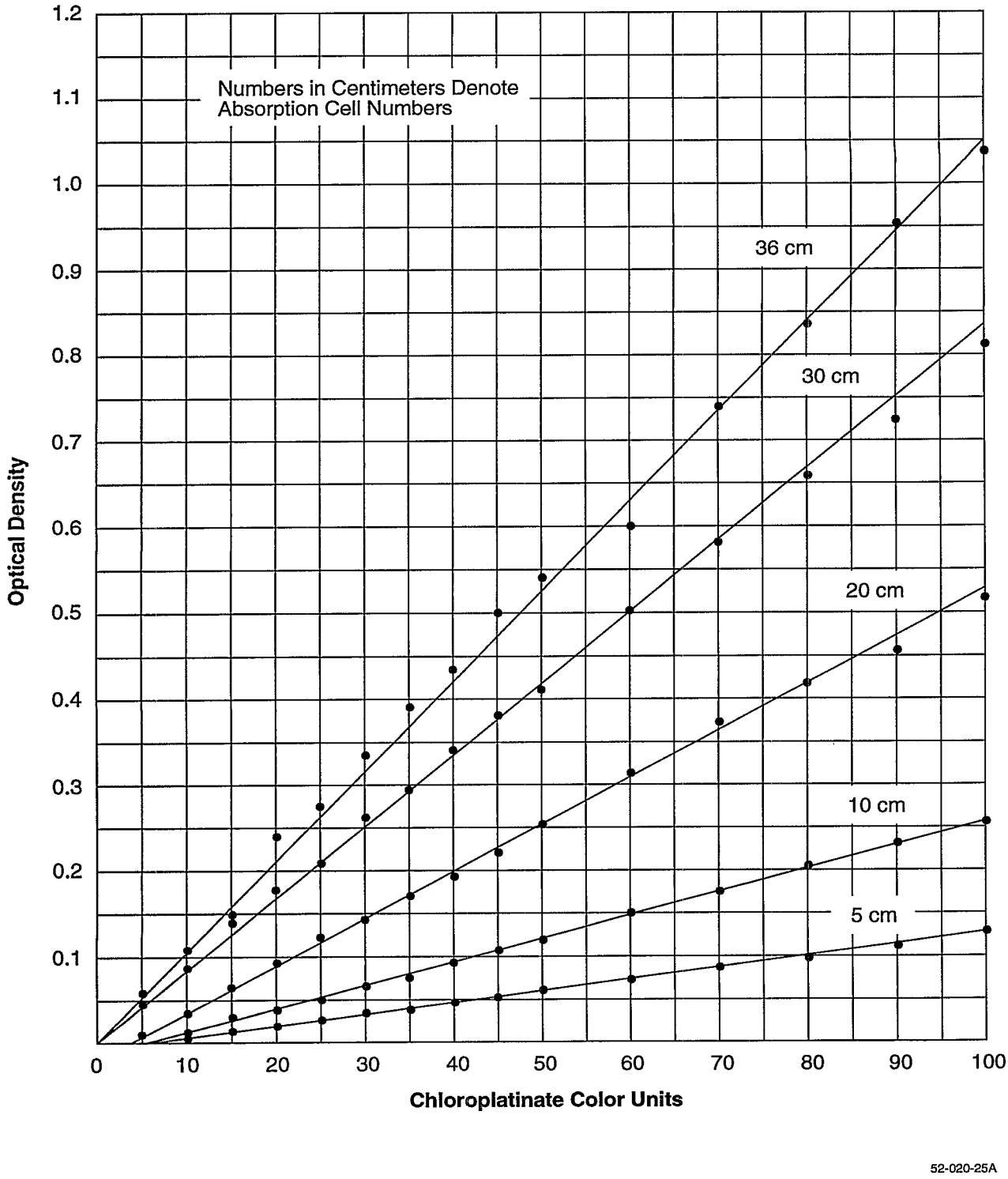


Figure 2. Typical Calibration Curves—Effect of Absorption Cell Length on Absorbance of Chloroplatinate Color Units