METHOD PC-97

DETERMINATION OF PHENOL AND CATECHOL IN WEAK AND STRONG BLACK LIQUORS, WASTEWATER TREATMENT PLANT INFLUENT, AND EFFLUENT FROM PULP AND PAPER MILLS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

NCASI
West Coast Regional Center
Organic Analytical Program
March 1997

Acknowledgements

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National Council of the Paper Industry for Air and Stream Improvement, Inc. (NCASI). 1997. Methods Manual, D*etermination of Phenol and Catechol in Weak and Strong Black Liquors, Wastewater Treatment Plant Influent, and Effluent from Pulp and Paper Mills by Gas Chromatography/Mass Spectrometry*, Research Triangle Park, N.C.: National Council of the Paper Industry for Air and Stream Improvement, Inc.

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DETERMINATION OF PHENOL AND CATECHOL IN WEAK AND STRONG BLACK LIQUORS, WASTEWATER TREATMENT PLANT INFLUENT, AND EFFLUENT FROM PULP AND PAPER MILLS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

1.0 Scope and Application

1.1 This method is designed to determine the concentration of catechol and phenol in weak and strong black liquor, wastewater treatment plant influent, and secondary biologically treated effluent samples from kraft, semichemical, and thermomechanical pulp and paper mills. This method is similar to NCASI Method CP-86.01, and involves derivatization of the analytes using base and acetic anhydride followed by extraction (NCASI 1986). Quantification by gas chromatography/mass spectrometry (GC/MS) using labeled catechol and phenol corrects for any matrix effects.

1.2 The compounds listed in Section 17, Table 1 may be determined in weak and strong black liquor, wastewater treatment plant influents, and effluents from kraft, semichemical, and thermomechanical pulp and paper mills.

1.3 This method has been validated at the single laboratory level, and is a proposed method.

1.4 The estimated method detection limits were determined as specified in 40 CFR 136 Appendix B (Federal Register 1984), using final effluent samples from two different mill sites. One of the mills was a complete chlorine dioxide substitution and oxygen delignification mill equipped with an aeration stabilization basin for secondary biological treatment. This mill produces bleached softwood kraft pulp. The calculated method detection limit was 1.1 and 0.5 µg/L for phenol and catechol, respectively, in the final effluent samples. The method detection limit was also determined in a second final effluent sample from an unbleached kraft mill equipped with an activated sludge system for biological treatment. This mill produces bags and multiwall sacks, laminating and other converting papers, and wrapping papers. The calculated method detection limit determined in this matrix was 0.6 and 1.0 µg/L for phenol and catechol, respectively. A method detection limit has not been determined in weak or strong black liquors or wastewater treatment plant influents. The lower instrument calibration limit is 1.6 µg/L for wastewater treatment plant influents, and 0.5 mg/L for strong and weak black liquors.

1.5 The GC/MS portions of this method are for use only by analysts experienced with GC/MS, or under the close supervision of such qualified persons.

2.0 Summary of Method

2.1 Weak black liquor

A 500 µL aliquot of weak black liquor (and the isotopically labeled analogs of phenol and catechol) are added to 300 mL of 0.1 M potassium phosphate dibasic (K_2HPO_4) buffer solution; and the solution is immediately spiked with a working stock (approximately 50 µg/mL) of phenol-d_5 and catechol-d_6. The target analytes are derivatized *in-situ* by the addition of acetic anhydride. The solution is extracted with hexane, and the extract is concentrated to a final volume of 0.5 mL. 2,2'-Difluorobiphenyl is added as an instrument internal standard, and the extract is analyzed by GC/MS.

2.2 Strong black liquor

Strong black liquor is very viscous and must be weighed out with the assistance of a clean spatula into a tared beaker. An aliquot of approximately 100 to 300 mg is weighed directly into the beaker. Three hundred milliliters of 0.1 M K_2HPO_4 buffer solution is added, and the solution is immediately spiked with a working stock (approximately 50 µg/mL) of phenol-d_5 and catechol-d_6, and stirred. The procedure mimics that of the weak black liquor from this point forward. The density of the black liquor samples is required to calculate the concentration of the target analytes. The density is determined empirically or by the graph in Figure 1.

2.3 Wastewater treatment plant influents and effluents

Three hundred milliliters of pH 2 preserved effluent is placed into a beaker, and spiked with a working stock (approximately 50 µg/mL) of phenol-d_5 and catechol-d_6. The pH is adjusted to approximately 7.5 with 1.8 M potassium phosphate tribasic (K_3PO_4). A solution of 1.7 M K_2HPO_4 is added, followed by acetic anhydride. The procedure mimics that for weak black liquor from this point forward. Influent is processed exactly as effluent, except that 150 mL of influent is added to a beaker and, after spiking with the working stock of the labeled compounds, 150 mL of reagent water is added to adjust the final volume to 300 mL.

2.4 Quantitative analysis

Quantitative analysis is performed by GC/MS, employing an isotope dilution technique using extracted ion current profile (EICP) areas. Identification of target analytes (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 meet the criteria described in Section 12.1.

2.5 Quality assurance

Quality is assured through reproducible calibration and testing of the extraction and GC/MS system. A method blank is analyzed with each sample set (samples started through the extraction process on a given day, to a maximum of 20), along with a sample duplicate and a matrix spike to ensure quality data.

3.0 Definitions

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

3.1.1 µg/L–micrograms per liter

3.1.2 mg/L–milligrams per liter

3.1.3 Acetylation–derivatization of the polar OH group with an acetyl group

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

3.1.5 May not–this action, activity, or procedural step is prohibited

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

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

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may contribute analytical interferences resulting in misinterpretation of chromatograms and spectra. All materials used in the analysis must be demonstrated to be free from interferences under the conditions of analysis by running method blanks initially and subsequently with each sample set. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

4.2 Interferences co-extracted from samples will vary considerably from source to source depending on the diversity of the site being sampled.

4.3 Catechol is susceptible to degradation by active sites on injection port liners and columns, and is subject to oxidation to the corresponding benzoquinone.

5.0 Safety

5.1 Many of the reagents used in this method are caustic; acetic anhydride is a lachrymator. They must be handled under a fume hood. Adequate eye protection, a lab coat, and gloves should be worn at all times.

5.2 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 a level protective of human health. 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.

5.3 As with all samples, precautions should be taken to avoid exposure to potentially toxic, caustic, or nuisance odor compounds, and should be handled with gloves and opened in a fume hood.

6.0 Equipment and Supplies

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

6.2 Do not use glassware with any star fractures, cracks, or severe scratches. All fittings should be snug, and clamps and springs should be in good working order. All glassware should be washed with detergent, rinsed with tap water, then rinsed with reagent-grade water.

6.3 Sampling equipment

Amber glass bottles equipped with Teflon™-lined screw caps are used to collect all samples.

6.4 Equipment for sample extraction

6.4.1 One 400 mL beaker (or larger) per sample, equipped with a Teflon™-coated stir bar

6.4.2 One 500 mL separatory funnel with ground-glass stopper and Teflon™ stop-cock

6.4.3 One 50 mL centrifuge tube with cap per sample

6.4.4 One graduated cylinder or autopipet (at least 10 mL) for acetic anhydride

6.4.5 One graduated cylinder large enough to measure 20 mL K_2HPO_4

6.4.6 One graduated cylinder large enough to measure 300 mL reagent water

6.4.7 One graduated cylinder large enough to measure 40 mL hexane

6.5 Equipment for sample concentration

6.5.1 15 mL, graduated concentrator tube, a ground-glass stopper may be used to prevent evaporation of extracts

6.5.2 250 mL evaporation flask

6.5.3 ½ inch springs

6.5.4 Three-ball macro Snyder column

6.5.5 One 2 mL glass autosampler vial with Teflon™-lined crimp top per sample

6.6 Other apparatus

6.6.1 Teflon™ boiling chips, and a hot water bath (in a hood) capable of ±5°C temperature control and preheated to a minimum of 95°C

6.6.2 A pH meter calibrated using a two-point calibration procedure and pH 7 and pH 10 buffers

6.6.3 Magnetic stirrer

6.6.4 Nitrogen evaporation apparatus

6.6.5 Balances–an analytical balance capable of weighing to the nearest 0.1 mg with an accuracy of ±0.1 mg, and a top loading balance capable of weighing to the nearest 10 mg with an accuracy of ±10 mg

6.6.6 Gas Chromatograph–must have a splitless injection port for capillary column, capable of running the temperature program and performance specifications outlined in Sections 9.2 and 10.1

6.6.7 Gas Chromatographic Column–30 ±5 m x 0.25 ±0.02 mm ID x 0.25 micron, 5% phenyl, 94% methyl, 1% vinyl silicone-bonded phase-fused silica capillary column (DB-5 or equivalent)

6.6.8 Mass Spectrometer–70 eV electron impact ionization, must repetitively scan from 42 to 420 AMU in 0.95 to 1.00 second, and must produce a unit resolution (valley 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 must meet the mass intensity criteria listed in Section 9.2 and Section 17, Table 2. The mass spectrometer must be interfaced to the GC so that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beam. All portions of the column which connect the GC to the ion source must remain at or above the oven temperature during analysis to preclude condensation of less volatile compounds.

6.7 The data system should collect and record the MS data, store the intensity data, process GC/MS data, generate reports, and compute and record response factors.

7.0 Reagents and Standards

7.1 Solvents

7.1.1 Hexane, methanol, and toluene supplied by Burdick & Jackson or equivalent high purity solvent suitable for gas chromatography and pesticide residue analysis

7.1.2 Organic free reagent water in which the compounds of interest and interfering compounds are not detected by this method. All organic-free water and buffer solutions should be stored in glass to prevent the leaching of contaminants from plastic containers. These glass containers must have tightly-fitting Teflon™-lined caps.

7.2 Standards

7.2.1 Phenol-d_5 98% pure and catechol-d_6 98% pure can be purchased from Cambridge Isotope Laboratories or an equivalent supplier.

7.2.2 Phenol 99+% pure and catechol 99+% pure can be purchased from Aldrich or an equivalent supplier.

7.2.3 The primary standards of phenol-d_5 and catechol-d_6 are prepared at a concentration of 10 mg/mL ± 1 mg in methanol. Place the solution into an amber glass vial with a Teflon™-sealed cap. Store the tightly-sealed standard stock solution at 4°C. The primary standard stock solution must be replaced if comparison with quality control check samples indicates a problem.

7.2.4 The primary standards of the phenol and catechol are prepared at a concentration of 10 mg/mL ± 1 mg in methanol. Place the solution into an amber glass vial with a Teflon™-sealed cap. Store the tightly-sealed standard stock solution at 4°C. The primary standard stock solution must

be replaced if comparison with quality control check samples indicates a problem.

7.2.5 Working stocks of phenol-d_5 and catechol-d_6 are prepared by diluting 500 µL of the primary stocks (Section 7.2.3) into a 100 mL volumetric flask with methanol, yielding a final concentration of approximately 50 µg/mL.

7.2.6 Working stocks of phenol and catechol are prepared by diluting 500 µL of the primary stocks (Section 7.2.4) into a 100 mL volumetric flask with methanol, yielding a final concentration of approximately 50 µg/mL.

7.2.7 Decafluorotriphenylphosphine (DFTPP) can be purchased from Supelco or an equivalent supplier as a 25,000 µg/mL solution in dichloromethane. A working stock solution at a concentration of 50 µg/mL is prepared in hexane. Store in the dark in autosampler vials with Teflon™-seal crimp caps prior to use.

7.2.8 2,2'-Difluorobiphenyl (DFB) 99% pure is available from Pfaltz and Bauer, Inc. or an equivalent supplier. A working stock solution of 2.0 mg/mL is prepared in toluene.

7.2.9 The method requires that the calibration standards be prepared by spiking the underivatized target analytes (Section 10.2.2) into reagent water and carrying them through the entire derivatization and extraction procedure that is applied to the samples. Standard solutions are prepared from materials that have a chemical purity of 98% or greater. If the chemical purity of any standard does not meet this requirement, the laboratory must correct all calculations, calibrations, and matrix spikes for the difference in purity.

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

7.3 Reagents for sample preservation and pH adjustment

Sulfuric acid (H_2SO_4), reagent grade, 6N in organic-free reagent-grade water.

7.4 Reagents for derivatization

7.4.1 The acetic anhydride (99%) can be purchased from Aldrich or a comparable supplier. The acetic anhydride must be fractionally-distilled twice prior to use to eliminate phenol as a contaminant.

7.4.2 Potassium phosphate, dibasic (K_2HPO_4), 98+% pure ACS reagent-grade can be purchased from Aldrich or a comparable supplier, and is used as supplied. Prepare a 1.7 M solution of K_2HPO_4 for use as a buffer.

7.4.3 Potassium phosphate, tribasic (K_3PO_4), 97% pure can be purchased from Aldrich or a comparable supplier, and is used as supplied. Prepare a 1.8 M solution of K_3PO_4 for use as a buffer.

8.0 Sample Collection, Preservation, and Storage

8.1 Wastewater treatment plant effluent and influent samples are collected in 1-L amber glass bottles with Teflon™-lined screw caps, and are preserved by acidification using sulfuric acid to pH 2 in the field. This will provide sufficient sample for analysis and supporting quality control testing. The samples are shipped with ice packs and are stored at a temperature of 4°C prior to extraction. The wastewater treatment plant influent and effluent samples are extracted within 30 days of collection. The extracts are maintained at a temperature of 4°C prior to analysis. The extracts are analyzed within 30 days of extraction.

8.2 Black liquor samples are collected in 30-mL amber bottles with Teflon™-lined screw caps. Prior to shipment, it is recommended that tape be wrapped around the bottle caps to seal the contents. Each sample is placed in a paint can packed with vermiculite, put into a foam cooler, and packed with ice to keep the sample cold during shipment. The shipping package should be labeled "This package conforms to the conditions and limitations specified in 49 CFR 173.4" (Federal Code of Regulations). The black liquor samples are extracted within 15 days of collection. Extracts are maintained at a temperature of 4°C prior to analysis. The extracts are analyzed within 30 days of extraction.

9.0 Quality Control

9.1 Each laboratory that uses this method should operate a formal quality assurance program. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.2 GC/MS performance

Prior to analyzing any samples or standards, the GC/MS performance must be verified by a DFTPP tune. This tune check must be analyzed just prior (±6 hours) to the calibration standard analyses, and must meet the specifications listed in Section 17, Table 2.

9.3 Calibration verification

Before each set of samples is analyzed, a calibration check will be done to determine that the GC/MS system is operating within acceptable parameters. The calibration check involves reanalyzing one of the extracts used in the calibration curve (Section 10.2.2). Analysis of different points from the curve over time will verify the full calibration range. Isotopic dilution with catechol-d_6 and phenol-d_5 as internal standards will be used to calculate the relative response of catechol and phenol, respectively. The calibration check is evaluated by calculating the relative response factor for each analyte based on the concentration of internal standard and the relative response factor of that internal standard. If the relative response factor determined for the calibration verification point analyzed varies by less than ±20% from the initial relative response factor determined for that point in the curve, the initial calibration curve may be considered valid. It appears that these analytes are very sensitive to GC/MS instrumental conditions such as incomplete source evacuation, or contamination of the injection port, column, and/or source, which becomes more evident as the m/z 69 ion increases. If the calibration check fails to meet the ±20% acceptance criterion, appropriate GC/MS maintenance is necessary. Upon completion of all necessary instrument maintenance, the calibration verification can then be reanalyzed. If all recommended instrument maintenance fails to correct all calibration verification difficulties, the calibration curve should be reanalyzed.

9.4 Blanks

Analysis of blanks is required to demonstrate that the analytical system is free of contamination. A blank sample is to be prepared with each group of samples. A method blank is prepared and analyzed using the same procedure as a regular sample. Place 280 mL of reagent water into a 400-mL beaker using a graduated cylinder. Immediately spike with 250 µL of approximately 50 µg/mL working stock of phenol-d_5 and catechol-d_6, and add 20 mL 1.7 M K_2HPO_4. Add 10 mL twice fractionally-distilled acetic anhydride and allow the solution to stir for ten minutes. Transfer the beaker contents quantitatively into a 500-mL separatory funnel and extract three times with 40 mL portions of hexane. Centrifuge the hexane layer and any emulsions after each extraction. Combine the three hexane extracts in a Kuderna-Danish apparatus, and concentrate to 0.5 mL. Add 10 µL of 2.00 mg/mL 2,2'-DFB, and transfer the final extract to an autosampler vial. Proceed with GC/MS sample analysis as described for the samples in Section 11.8.

9.5 Internal standard recovery spikes

The laboratory must spike all samples with the labeled compounds to monitor internal standard recovery. Compute the recovery of each of the labeled compounds as the ratio of concentration found to the concentration spiked, using Equation 1.

Equation 1 $$\textit{Percent recovery} = \frac{\textit{Concentration found x 100}}{\textit{Concentration spiked}}$$

The criteria for acceptable labeled internal standard recoveries is the average recovery ±two times the standard deviation of the recoveries. If the recovery is greater or less than the acceptable criteria action should be taken to resolve the problem and the samples should be reextracted and reanalyzed. The percent recovery and related statistics for the labeled internal standards determined in a single laboratory validation of this method are presented in Section 17, Table 3.

9.6 Matrix spikes

With each set of samples, a matrix spike will be analyzed to assess the accuracy of the method. Black liquor consistently contains phenol and catechol at varying concentrations, so generally only one sample is required to be matrix spiked per sample batch. Wastewater treatment plant effluent and influent samples may contain low levels of analytes, therefore a duplicate matrix spike is appropriate. A 500 µL spike of the unlabeled working stock (Section 7.2.6) should be added to the sample prior to derivatization. The amount of unlabeled working stock to add should be adjusted to give a final concentration in the sample that is a minimum of twice the native level in the sample. Prepare the matrix spike sample in exactly the same manner as a regular sample, using the acetylation, extraction, and concentration procedures outlined in Section 11.0. An acceptable matrix spike recovery is the average recovery ±two times the standard deviation of the recoveries. If the matrix spike recoveries are greater or less than the average recovery ±three times the standard deviation, action should be taken to resolve the problem and the samples should be reextracted and reanalyzed. The average recoveries determined in a single laboratory validation study and the observed relative standard deviations are reported in Section 17, Table 4.

9.7 Sample and duplicate precision

Each set of samples will also include the analysis of a sample and duplicate to assess the precision of the analyses. For effluent and influent samples that may contain low levels of analytes, a duplicate matrix spike can be used to assess precision. The relative percent difference in concentration is calculated for each sample and duplicate pair by using Equation 2.

Equation 2

$$\textit{Relative Percent Difference} = \frac{(\textit{Highest concentration} - \textit{Lowest concentration}) \times 100}{\textit{Average concentration of the samples and duplicate}}$$

A summary of the precision determined in a single laboratory is provided in Section 17, Table 5, for wastewater treatment plant influents and black liquor samples. The native analyte precision was not determined in effluent samples, since the compounds were frequently not detected.

9.8 Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10.0 Calibration and Standardization

10.1 Assemble the GC/MS and establish the operating conditions outlined below. Optimize the GC conditions for analyte separation and sensitivity. Once the GC system is optimized, the same operating conditions must be used to analyze all samples, blanks, calibration curves, calibration verification samples, and matrix spikes.

GC/MS Operating Conditions for NCASI Method PC-97

Injector Temperature:	210°C
Splitless Valve Time:	1.0 min
Carrier Gas:	Helium @ 30 cm/sec & 23°C
Injection Volume:	1 μL

Temperature Program °C:

Initial:	50 for 1 min
Ramp:	50 to 160 @ 12°C/min
Final:	160 for 2 min
Ramp 2:	160 to 300 @ 35°C/min
Post Run:	300 for 4.8 min
Oven Equilibration:	0.50 min
Run Time:	21.0 min
Interface Temperature:	290°C

MS Conditions:

Scan Start Time:	4.00 min
Scan Range:	42 to 420 AMU
Cycle Time:	0.95 -1.00 seconds

10.2 Preparation of the calibration curve

10.2.1 Prepare a six point calibration curve encompassing the concentration range for each of the target compounds. The concentration ranges for the method for phenol and catechol are as follows.

Concentration Ranges for NCASI Method PC-97

Effluent	0.8 to 167 µg/L
Influent	1.6 to 333 µg/L
Black Liquor	0.5 to 100 mg/L

10.2.2 Prepare the calibration curve in the following manner. Each calibration standard is prepared, spiked, and derivatized sequentially, i.e., one at a time. Immediately add 5 µL of the unlabeled phenol and catechol working stock solution (Section 7.2.6) via a micro-liter syringe, and 20 mL 1.7 M K_2HPO_4, into a 280-mL aliquot of reagent water in a 400-mL beaker equipped with a Teflon™-coated stir bar. The curves for weak and strong black liquor, influent, and effluent will require the same amount of working stock, but the curve is quantitated by the GC/MS operator, based on the sample size used in the study.

Sample Size

Black Liquor	Effluent	Influent
µL	300 mL	150 mL

Immediately add 250 µL of the labeled internal standard working stock (Section 7.2.5, approximately 50 mg/mL) via micro-liter syringe.

Concentration in 500 µL black liquor: *ca.* 25.0 mg/L
Concentration in 300 mL effluent: *ca.* 42.0 µg/L
Concentration in 150 mL influent: *ca.* 83.0 µg/L

Add 10 mL of acetic anhydride as quickly as possible and stir for ten minutes. Transfer the 300 mL solution to a 500-mL separatory funnel for extraction.

Repeat this process using 10, 50, 250, 500 and 1000 µL of the unlabeled working stock solution (Section 7.2.6), to result in a six-point calibration curve. Extract the derivatized calibration points by adding 40 mL of hexane into each separatory funnel, and allowing the mixture to settle for 30 seconds. Seal the separatory funnel by holding the ground glass stopper quite firmly, shake once, and immediately vent. Continue to shake the separatory funnel vigorously for two minutes, with periodic venting. Allow the organic (bottom) layer to separate from the water phase for a minimum of five minutes. Drain the water layer into the 400-mL beaker which previously held the sample. Drain the hexane layer into a centrifuge tube, and cap. Place the capped tube into the centrifuge and spin at full speed for three to five minutes. Pipet the top hexane layer into a KD apparatus. Pour the water layer back into the separatory funnel to be re-extracted. Repeat the extraction procedure two more times using fresh

40 mL portions of solvent, combining the three solvent extracts in the KD. Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding 1 mL of hexane to the top of the column. Place the KD apparatus into a hot water bath at a minimum temperature of 95°C. When the apparent volume of liquid reaches 5 mL, remove the KD apparatus from the water bath, and allow it to drain and cool. Remove the Snyder column, and rinse the flask and its lower joints into the concentrator tube with 1 to 2 mL of hexane. Preheat the nitrogen blowdown waterbath to a minimum of 60°C. Place the concentrator tube in the apparatus and carefully evaporate the solvent volume to 0.5 mL using a gentle stream of clean, dry nitrogen. Add 10 µL of 2,2'-DFB (Section 7.2.8). Using a disposable pipet, transfer to a labeled vial with a tightly-sealed Teflon™-lined screw cap or crimp top. Store the extracts at 4°C prior to analysis. The nominal 50 µg/mL calibration curve extract may be used as a calibration verification standard (Section 9.3).

10.3 Isotopic dilution calibration

10.3.1 Assess isotopic purity of the native and labeled materials prior to development of a calibration curve by a separate analysis of the two materials. Two extracts are prepared for this purpose. Fortify a 300 mL portion of reagent water with phenol-d_5 and catechol-d_6 by spiking 250 µL of the approximately 50 µg/mL working stocks into the reagent water. Carry this solution through the calibration standard preparation procedure (Section 10.2.2), and analyze according to Section 11.8. Fortify an additional portion of reagent water with the native phenol and catechol at the same concentration as the midpoint of the calibration range specified in Section 10.2, i.e., 500 µL of approximately 50 µg/mL solutions. Carry this solution through the calibration standard preparation procedure (Section 10.2.2), and analyze according to Section 11.8. From analysis of these two extracts, and use of Equations 3 and 4, isotopic dilution correction factors (R_x and R_y) are obtained.

Equation 3

R_x = [area (m_1/z)] / [area (m_2/z)] from analysis of the phenol and catechol extract

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Equation 4

R_y = [area (m_1/z)] / [area (m_2/z)] from analysis of the phenol-d_5 and catechol-d_6 extract

where:

(m_1/z) is the ion that is used to quantify the phenol or catechol, and (m_2/z) is the ion used to quantify the phenol-d_5 or catechol-d_6. If any of the measured area counts are zero, they are assigned a value of one in the calculations. The values of R_x and R_y need to be determined only once for each batch of unlabeled and labeled solutions (Section 7.2.5 and 7.2.6), and R_x must always be greater than R_y.

10.3.2 For every calibration standard analyzed, the relative response (RR) of unlabeled to labeled compound is calculated.

Equation 5

$$RR = [(R_y - R_m)(R_x + 1)] / [(R_m - R_x)(R_y + 1)]$$

where:

R_m = [area (m_1/z)] / [area (m_2/z)] from analysis of the calibration standard and R_x and R_y are as defined in Section 10.3.1. If R_m is not between $2R_y$ and $0.5R_x$, isotopic dilution quantification cannot be applied. It is recommended that the purity of the standards be assessed, and the standards be replaced if impurities are indicated. The blank should also be evaluated to determine if phenol levels are high. This can indicate that the acetic anhydride needs further purification. The calibration curve will need to be prepared and analyzed using the new standards and reagents if impurities are detected.

10.3.3 For every calibration standard analyzed, the isotopic dilution relative response factor (RRF_{ID}) is then calculated using Equation 6.

Equation 6

$$RRF_{ID} = RR (C_L/C_N)$$

where:

RR = the extract-specific relative response calculated in Section 10.3.2
C_L = the extract-specific concentration of the phenol-d_5 or catechol-d_6
C_N = the extract-specific concentration of the phenol or catechol

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

10.3.4 If the area at m_2/z in the determination of R_x is zero *and* the area at m_1/z in the determination of R_y is also zero, isotopic corrections are not necessary and R_m = RR (Section 10.3.2). Under these conditions, quantifications can be performed by the internal standard method using the labeled compounds as internal standards for the corresponding unlabeled analytes to determine the relative response factors (Section 10.4).

10.3.5 If $R_x > 1000$ *and* $R_y < 0.001$, isotopic corrections are not necessary and R_m approaches RR (Section 10.3.2). Under these conditions, quantifications can be performed by the internal standard method using the labeled analogs as the internal standard (Section 10.4).

10.4 When isotopic corrections are not necessary, the relative response factors are calculated using Equation 7.

Equation 7

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

where:

A_S = *area of the unlabeled compound's characteristic mass in the calibration standard*
A_{IS} = *area of the characteristic mass for the labeled compound, internal standard*
C_S = *concentration of the unlabeled compound in the calibration standard*
C_{IS} = *concentration of labeled compound (internal standard) in the calibration standard*

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

10.5 The instrument internal standard (2,2'-DFB) is used for determining the recovery of the labeled internal standards. The labeled internal standard recoveries are used to assess quality control as described in Section 9.5. Internal standard quantification of

the labeled isotopic dilution internal standards requires the determination of relative response factors (RRF) versus the instrument internal standard 2,2'-DFB:

Equation 8

$RRF = [(A_L/A_{IIS})(C_{IIS}/C_L)]$

where:

A_L = *area of labeled compound's characteristic mass from analysis of calibration standard*
A_{IIS} = *area of 2,2'-DFB characteristic mass from analysis of calibration standard*
C_L = *concentration of labeled compound in calibration standard*
C_{IIS} = *concentration of 2,2'-DFB in calibration standard*

The concentration of the 2,2'-DFB remains constant in each of the six points of the calibration curve prepared in Section 10.2.2, as does the labeled compound concentration. If the relative response factors are constant (less than 20% relative standard deviation) over the six replicates, an average relative response factor is used for that compound. If the relative response factors vary by more than 20%, the curve is reprepared and reanalyzed.

10.6 Verify the calibration curve prior to the analysis of each set of samples (Section 9.3). Recalibration is required if the relative response factor for the target compounds in the calibration verification point analyzed differs by ±20% of the relative response factor determined for that calibration point in the current calibration curve.

10.7 Process a blank with the curve as specified in Section 9.4 to confirm that the glassware, reagents, etc. are free from contamination.

10.8 Analytical range and minimum calibration level

10.8.1 Demonstrate that the calibration curve is linear (relative response factors exhibit a coefficient of variation < 20%) throughout the range of the calibration curve.

10.8.2 Demonstrate that the target analytes are detectable at the minimum levels using the lowest level calibration curve extract.

11.0 Procedures

11.1 This section includes the procedures used to derivatize, extract, and concentrate weak and strong black liquor, influent, and effluent samples. The derivatization procedure applied to the samples depends on the type of sample being analyzed. The same extraction and concentration procedures are used for all types of samples. Remove the sample, catechol-d_6 and phenol-d_5 working stock (Section 7.2.5), 2,2'-difluorobiphenyl working stock (Section 7.2.8), and the appropriate catechol and

phenol matrix spike/working stock solution (Section 7.2.6) from the refrigerator, and bring to room temperature. Shake the sample to ensure homogeneity.

11.2 Acetylation of weak black liquor samples

Measure 280 mL of reagent water into a graduated cylinder, and add 20 mL 1.7 M K_2HPO_4 to give a 0.1 M pH 9.2 buffer solution. Pour into a 400-mL beaker equipped with a Teflon™-coated stir bar. While stirring, add 500 µL of weak black liquor sample by microliter syringe, placing the tip of the needle into the buffer solution. Immediately add 250 µL of the catechol-d_6 and phenol-d_5 working stock via microliter syringe. If the sample is to be a matrix spike, the phenol and catechol matrix spike/working stock is immediately added to the sample at this time. Add 10 mL of acetic anhydride by autopipet or graduated cylinder, and allow the mixture to react for ten minutes. Transfer the solution into a 500-mL separatory funnel.

11.3 Acetylation of strong black liquor

With a spatula, scoop out some strong black liquor and smear enough onto the base of the 400-mL tared beaker to accumulate approximately 0.15 to 0.20 g. Reweigh the beaker on a top-loading balance and record the strong black liquor weight to ±10 mg. Measure 280 mL of reagent water into the beaker and add 20 mL 1.7 M K_2HPO_4. Immediately thereafter, add 250 µL of the catechol-d_6 and phenol-d_5 working stock via microliter syringe. Simultaneously, add the appropriate amount of catechol and phenol matrix spike/working stock if the sample is to be a matrix spike sample. Immediately add a Teflon™-coated stir bar and stir rapidly until the strong black liquor is dissolved. Add 10 mL of acetic anhydride by autopipet or graduated cylinder, and allow the mixture to react for ten minutes. Transfer the solution into a 500-mL separatory funnel.

11.4 Acetylation of effluent samples

Measure a 300 mL effluent sample into a 400-mL beaker equipped with a Teflon™-coated stir bar. While stirring, add 250 µL of the catechol-d_6 and phenol-d_5 working stock via microliter syringe. Immediately add the designated amount of the phenol and catechol matrix spike/working stock to the sample selected as the matrix spike. Carefully add 1.8 M K_3PO_4 by disposable pipette to bring the solution to pH 7.5 ± 0.1. Add 20 mL 1.7 M K_2HPO_4. Add 10 mL of acetic anhydride by autopipet or graduated cylinder, and allow the mixture to react for ten minutes. Transfer the solution into a 500-mL separatory funnel.

11.5 Acetylation of influent samples

Measure 150 mL of the influent sample into a 400- mL beaker equipped with a Teflon™-coated stir bar. While stirring, add 250 µL of the catechol-d_6 and phenol-d_5 working stock via microliter syringe. Immediately add the designated amount of the phenol and catechol matrix spike/working stock to the sample selected as the matrix

spike. Adjust the total volume to 300 mL by adding 150 mL of reagent water. Carefully add 1.8 M K_3PO_4 by disposable pipette to bring the solution to pH 7.5 ±0.1. Add 20 mL 1.7 M K_2HPO_4. Add 10 mL of acetic anhydride by autopipet or graduated cylinder, and allow the mixture to react for ten minutes. Transfer the solution into a 500-mL separatory funnel.

11.6 Extraction

Add 40 mL hexane to the funnel. Allow the hexane to settle for 30 seconds. Cap the separatory funnel, shake, and vent over a period of two minutes. Allow the organic (bottom) layer to separate from the water phase for a minimum of five minutes. Drain the water layer into the 400-mL beaker which previously held the sample. Drain the hexane layer into a centrifuge tube, and cap. Centrifuge the extract at full speed for two to five minutes. Pipet the top layer into a KD apparatus, and pour the aqueous phase back into the separatory funnel along with the aqueous portion in the 400-mL beaker. Repeat the extraction procedure two more times, using fresh 40 mL portions of hexane. Combine the three solvent extracts.

11.7 Concentration

Check to confirm that the water bath temperature is at a minimum of 95°C. Add a clean boiling chip to the apparatus, and attach a 3-ball Snyder column. Pre-wet the Snyder column by adding 1 mL of hexane to the top of the column. When the apparent volume of liquid reaches 5 mL, remove the KD apparatus from the water bath, and allow it to drain and cool. Remove the Snyder column, and rinse the flask and its lower joints into the concentrator tube with 1 to 2 mL of hexane. Preheat the nitrogen blowdown waterbath to a minimum of 60°C. Place the concentrator tube in the apparatus, and carefully evaporate the solvent volume to 0.5 mL using a gentle stream of clean, dry nitrogen. If the volume of solvent is reduced below 0.5 mL during blowdown, analytes may be lost. Add 10 µL of the 2,2'-DFB working stock. Using a disposable pipet, transfer to a labeled vial with a tightly sealed Teflon™-lined crimp top. If stored, keep at 4°C.

11.8 GC/MS analysis

11.8.1 The GC/MS conditions should be set according to conditions described in Section 10.1.

11.8.2 Bring the DFTPP tune and the daily calibration check solution to room temperature. Perform the DFTPP tune (Section 9.2) and the GC/MS calibration check as outlined in Section 9.3.

11.8.3 Bring the sample extract or standard to room temperature and verify that any precipitate has redissolved. Inject 1 µL volume of the standard solution or extract using on-column splitless injection. The relative retention times of each analyte are based upon that of the instrument internal standard 2,2'-

DFB. The concentration of the instrument internal standard 2,2'-DFB is based upon the sample size, and will be approximately 40.0 mg/L in black liquor samples, 66 µg/L in effluents, and 132 µg/L in influents. The labeled internal standard concentrations are also based on the sample size, and are approximately 25 mg/L in black liquor samples, 42 µg/L in effluents, and 83 µg/L in influents.

12.0 Data Analysis

12.1 An analyte is identified by comparison of the sample mass spectrum with the mass spectrum of a standard of the suspected compound which has been previously stored in a mass spectral library. Refer to Section 17, Table 6 for a list of the characteristic ions. Identification of a compound is confirmed when the following criteria are met:

12.1.1 All characteristic m/z signals stored in the spectral library are present and maximize within the same two consecutive scans.

12.1.2 The sample component relative retention time (RRT) must fall within ±0.06 RRT units of the RRT of the standard component.

12.1.3 The relative percent abundance of the ions designated in Section 17, Table 6 must agree within ±20% between those observed in the calibration standards and those observed in the sample extracts.

12.2 The RRT should be assigned by using EICPs for ions unique to the component of interest.

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

12.4 Internal standard quantitation

12.4.1 When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. Quantitation will take place using both the internal standard and the isotopic dilution technique.

12.4.2 The labeled internal standards are used to quantitate the corresponding unlabeled compounds. The concentration of the compound in the sample is calculated according to Equation 9:

Equation 9

Concentration of target (mg/L or mg/L) = $[(A_s \times C_L)/(A_L \times RRF_{ID})]$

where:

A_s = Area of characteristic ion for the compound being measured
C_L = Concentration (mg/L or mg/L) of the labeled compound in the sample
A_L = Area of characteristic ion of the labeled internal standard
RRF_{ID} = Averaged relative response from the initial calibration using isotopic correction (if isotopic correction is not necessary, RRF_L is substituted)

12.4.3 The concentration of the target analytes in the strong black liquor solids must be corrected to reflect the concentration of the compound of interest in the mass of sample extracted. This is accomplished using Equation 10:

Equation 10

$C_{SBL} = [C_{MS} \times (V/ (M / D))]$

where:

C_{SBL} = Concentration (mg/L or mg/L) of target analyte in strong black liquor
D = Density of the strong black liquor in g/mL as determined empirically or from Figure 1
V = 0.5 mL
M = Mass of strong black liquor extracted in grams
C = Concentration (mg/L or mg/L) from Section 12.4.2

12.4.4 The instrument internal standard 2,2'-DFB is used to quantitate the labeled compounds. The concentration of the labeled standards using 2,2'-DFB are used to calculate the percent recovery of the labeled compounds and to track method performance (Section 9.5). The concentration of the labeled compounds is determined using 2,2'-DFB as the internal standard by Equation 11.

Equation 11

Concentration of labeled analyte (mg/L or mg/L) = [(A_L x C_{IS})/(A_{IS} x RRF)]

where:

A_L = Area of characteristic ion for the labeled analyte
C_{IS}= Concentration (mg/L or mg/L) of the 2,2'-DFB instrument internal standard in the sample
A_{IS} = Area of characteristic ion of the 2,2'-DFB instrument internal standard
RRF = Averaged response factor from the initial calibration using 2,2'-DFB as the internal standard

12.5 Data review requirements

12.5.1 The data is reviewed for accuracy of the identification, GC problems, interferences, and bias. Any problems should be corrected prior to reporting the analytical results.

12.5.2 All the chromatograms are manually reviewed to confirm internal standard and analyte identification and area integrations. As part of this review, the analyst will assess the need for sample/extraction dilutions. The procedure for conducting extract dilution and reanalysis is described in Section 12.6.

12.5.3 The area counts of the 2,2'-DFB internal standard are checked over the entire batch of samples to see if loss of sensitivity is occurring over time. The total ion chromatogram is visually inspected for obvious problems which might result in poor labeled internal standard recoveries or false negatives/false positives. From this review, the presence of non-target species can become apparent.

12.5.4 Any inconsistencies between duplicate analyses are resolved (i.e. if a compound shows up in one replicate but not the other), and attempts are made to determine the reason.

12.5.5 A GC/MS report is generated that includes the retention time of the compound, area of the compound, and calculated concentration of the target compound detected. If review of the data shows any problems which could affect subsequent analyses, analyses are discontinued until the problems are resolved.

12.6 Results outside the calibration range

If the EICP area at the quantitation m/z for any compound exceeds the area of the quantitation m/z of the highest calibration, dilute an aliquot of the extract 1:10 with hexane and run a separate analysis. Place an insert into an autosampler vial and fill with 90 µL of hexane using a microliter syringe. Using a 10 µL microsyringe, draw up 10 µL of the extract, and add to the 90 µL hexane, placing the tip of the syringe into the solvent. Since the internal standard is also diluted, no correction of the analytical results is required.

13.0 Method Performance

13.1 Single laboratory performance for this method is detailed in Section 17, Tables 3, 4, and 5.

13.2 A chromatogram of a calibration check standard is shown in Section 17, Figure 2.

14.0 Pollution Prevention

Pollution prevention approaches were not evaluated for this method.

15.0 Waste Management

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

15.2 Instructions for sample and waste handling and disposal

15.2.1 All flammable waste solvents are stored in a metal safety can labeled FLAMMABLE until proper disposal can be accomplished.

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

15.2.3 The potassium dibasic solution is approximately pH 9.2, and may be poured down the drain with copious amounts of water. The potassium tribasic solution may be neutralized and poured down the drain with copious amounts of water.

15.2.4 The aqueous portion of the extracted sample aliquot may be poured down the drain with copious amounts of water.

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

16.0 References

16.1 Code of Federal Regulations 49 CFR 173.4.

16.2 Federal Register, Vol. 49, No. 209. October 26, 1984. *Appendix B to Part 136-Definition and procedure for the determination of the method detection limit-revision 1.11*.

16.3 National Council of the Paper Industry for Air and Stream Improvement, Inc. (NCASI). 1986. *Chlorinated phenolics in water by in situ acetylation/GC/MS determination, Method CP-86.01*. Technical Bulletin No. 498. Research Triangle Park, NC: National Council of the Paper Industry for Air and Stream Improvement, Inc.

16.4 Technical Association of the Pulp and Paper Industry (TAPPI). 1940. Data Sheet Number 58. Issued December 1940. New York, New York.

16.5 Taylor, J.K. 1989. *Quality assurance of chemical measurements*. Chelsea, Michigan: Lewis Publishers.

17.0 Tables, Diagrams, Flow Charts, and Validation Data

Table 1 Compounds Determined by GC/MS Using NCASI PC-97

Compound	CAS Registry Number
Catechol	120-80-9
Phenol	108-95-2

Table 2 DFTPP Criteria for NCASI PC-97

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

Table 3 Labeled Internal Standard Recovery During Method Validation Studies

Compound	Spike Concentration	Recovery Range	Average Recovery	Relative Standard Deviation	RSD	n
		(%)	(%)		(%)	
Effluents	(µg/L)					
Catechol-d_6	43.2	62 - 107	88.1	10	11	69
Phenol-d_5	48.8	71 - 122	103	10	9.8	69
Influents						
Catechol-d_6	86.3	64 - 114	89.1	9.0	10	79
Phenol-d_5	97.6	56 - 123	96.3	13	13	79
Strong Black Liquor	(mg/L)					
Catechol-d_6	25.9	63 - 134	95.7	14	19	11
Phenol-d_5	29.3	70 - 133	94.9	15	17	11
Weak Black Liquor						
Catechol-d_6	25.9	81 - 137	114	17	13	21
Phenol-d_5	29.3	74 - 129	99.9	17	15	21

Table 4 Matrix Spike Recovery for Unlabeled Compounds During Method Validation Studies

Compound	Spike Concentration	Recovery Range	Average Recovery	Standard Deviation	RSD	n
		(%)	(%)		(%)	
Effluents	(µg/L)					
Catechol	43.3	90 - 119	106	7.3	6.9	11
Phenol	42.0	100 - 110	106	2.8	2.7	11
Influents						
Catechol	43.3	89 - 115	102	8.3	8.1	7
Phenol	42.0	100 - 110	106	3.5	3.3	7
Black Liquor	(mg/L)					
Catechol	25.9	45 - 116	91.2	14	16	6
Phenol	29.3	50 - 111	83.3	18	22	6

Table 5 Single Laboratory Precision of Native Analytes

Compound	Native Concentration Range	Relative Percent Difference Range	Mean RPD	Standard Deviation[1]	n
		(%)	(%)		
Influents	(µg/L)				
Catechol	2.2 - 22	0.4 - 7.5	4.2	2.0	6
Phenol	0.6 - 31	1.6 - 4.1	9.0	9.8	7
Black Liquor	(mg/L)				
Catechol	29 - 84	0.4 - 24	6.0	8.5	7
Phenol	3.4 - 49	0.2 - 13	6.2	3.8	8

1 = Taylor, 1989

Table 6 Characteristic Ions for NCASI PC-97 Compounds

Compound	Primary Ion	Secondary Ions
Catechol	110	152
Phenol	94	136, 66
Catechol-d6 (IS)	114	156, 113
Phenol-d5 (IS)	99	141, 71
2,2'-DFB (IS)	190	191, 170

IS = Internal Standard

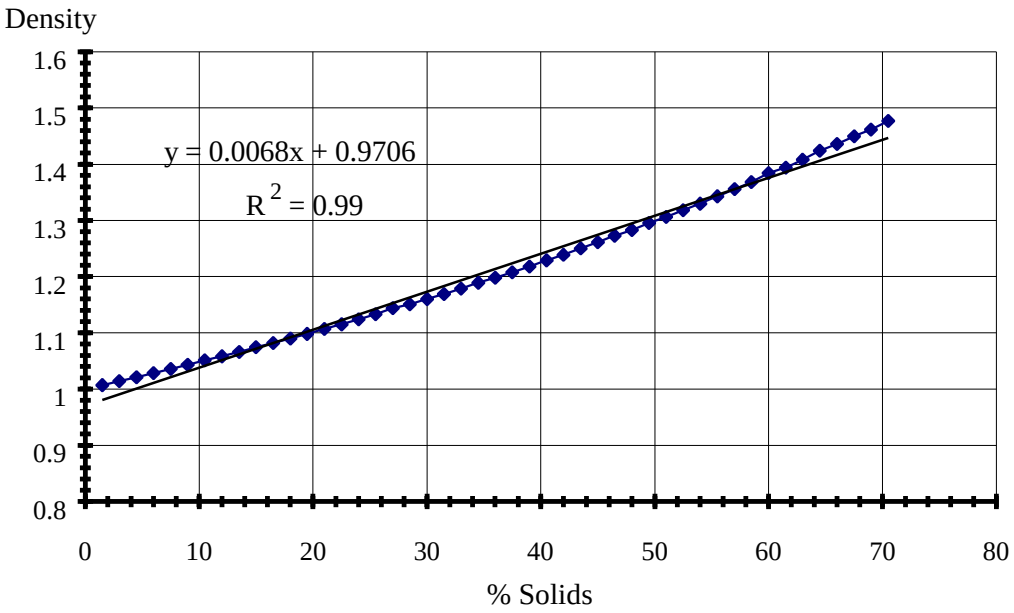

Figure 1 Density versus (%) Solids Graph for Black Liquor Samples at 60 °F (TAPPI 1940)

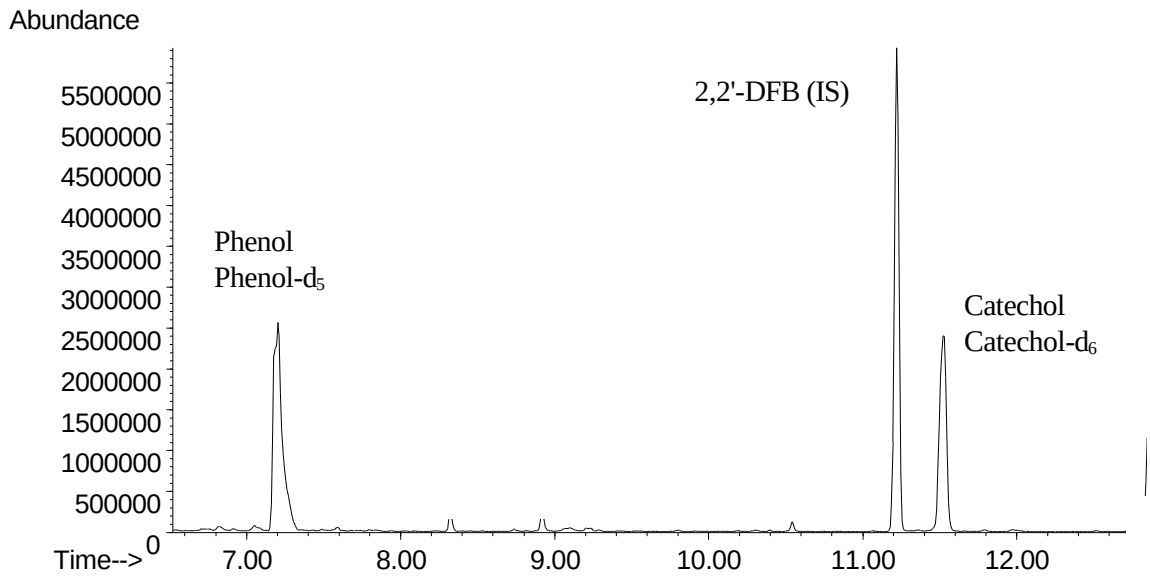

Figure 2 Chromatogram of the Calibration Check Standard