Development and validation of analytical methods for the determination of phenolic compounds in pulp and wastewater treatment plant sludges

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ABSTRACT: Procedures for the determination of phenolic compounds in bleached pulp and wastewater treatment plant sludges have been investigated. From the results of survey experiments, Soxhlet extraction with ethanol/toluene (68:32 v:v %) was selected for extraction of the tested matrices. Addition of 70 mL reagent water to the resulting ethanol/toluene extract followed by a Kuderna-Danish concentration gives an aqueous phase which is acetylated in situ. The hexane extract from the acetylation is then subjected to a silica gel cleanup and analyzed by full scan GC/MS. Single laboratory precision for the method is presented by matrix type. Nominal full scan MS detection limits of 25 ppb (µg/kg wet weight) to 500 ppb (µg/kg dry weight) can be achieved with the reported method. Results from a sample storage stability study testing freezing at –15° C suggest a maximum storage time for sludges of five months.

KEYWORDS: Analysis, aromatic compounds, bleached pulps, chemical analysis, chemical tests, chlorine compounds, development, effluent treatment, effluents, evaluation, halogen compounds, phenols, pulps, sludge, solid wastes, test methods, wastewaters, water.

The literature holds a number of references to analytic methods for determining phenolics in riverine or marine sediments *(1–3)*. However, very little work has been reported on the determination of the same species in pulp or in pulp and paper industry wastewater treatment plant sludges. In order to provide an analytical method suitable for routine support of the NCASI research program, studies were undertaken to evaluate suitable analytical procedures for these matrices.

At the onset, the distinction between "free" and "bound" moieties was recognized. As defined by Remberger *et al. (1)*, "free" moieties are those recoverable directly by solvent extraction, while "bound" moieties can only be recovered by more rigorous chemical treatment (i.e., digestion). Based on a review of NCASI research objectives, it was determined that a validated "nonaggressive" procedure for characterization of "free" phenolics was of highest priority. Such a method provides the foundation necessary for further investigations of analytical approaches to the determination of "bound" moieties. Throughout the process of method development, appropriate ruggedness studies were undertaken to assure that the final method could routinely provide reliable data at the 100 µg/kg level (wet weight basis).

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Experimental

Standards

Phenol, *o*-, *m*-, and *p*-cresol, guaiacol, 4-chlorophenol, 2,6-dichlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, 3,4,5-trichlorophenol, pentachlorophenol, 2-fluorophenol, and 2,4,6-tribromophenol were obtained from Aldrich (Milwaukee, WI); 2,3,4,6-tetrachlorophenol was obtained from Ultra (N. Kingstown, RI); 5-chlorovanillin was obtained from Pfaltz & Bauer (Waterbury, CT); 2-chlorophenol-d_4 was obtained from Cambridge Isotopes (Woburn, MA); 3,5-dichloro-4-hydroxybenzaldehyde was synthesized according to Neilson *(4)*. A tribromoguaiacol was prepared by the dropwise addition of bromine to a chloroform solution of guaiacol at

room temperature. The crude reaction mixture was poured into water and extracted three times with methylene chloride. After removal of the solvent, the product was recrystallized from toluene. All other compounds were obtained from Helix Biotech (Richmond, BC, Canada).

Sample preparation

Prior to analysis, all samples were homogenized. Combined dewatered sludges and pulps were manually broken into "small" pieces (*ca.* 0.125 in. in diameter) and mixed; pulps were treated in the same manner. Secondary sludges were first centrifuged, and the supernatant discarded; the remaining sample was stirred to homogenize. Typically, combined dewatered sludges had consistencies ranging from 35% to 50% solids, pulps ranged from *ca.* 7% to 20% solids, and secondary sludges were *ca.* 3–7% solids (after the initial centrifuge step).

Soxhlet extractions

Soxhlet extractions were carried out using a nominal 30 g (wet weight) sample size for combined dewatered sludges and bleached pulps. Samples were placed in a Soxhlet thimble (45 x 130 mm, extra coarse frit) containing a layer of silica gel (5 g), a plug of glass wool was placed on top of the sample, and any surrogate spikes added into/onto the sample. For secondary sludges, a nominal 10 g portion of the centrifuged sample was mixed with 125 g silica sand and transferred to a Soxhlet thimble as above. All extractions were performed without pH adjustment using 300 mL of the respective solvent for 24 h. Solvents tested were: acetone, acetonitrile, methylene chloride, ethanol/toluene (68:32% V:V%), acetone/hexane (60:40 V:V%), isopropanol/hexane (22:78 V:V%), acetic acid/hexane (6:94 V:V%), and acetonitrile/methanol (81:19 V:V%). After 24 h, the solvent was collected and concentrated to 10 mL (nominal). Concentration was accomplished by adding Teflon™ boiling chips to the Soxhlet round bottom flask, attaching a Snyder column, and

I. Characteristic m/z's used for qualitative identification and quantitation (base peak) of phenolics, and relative retention times vs. the injection internal standard 2,2'-difluorobiphenyl.

	RRT [a]	*Base peak* [c]	*Confirmatory ion 1, %*	*Confirmatory ion 2, %*
2,2'-Difluorobiphenyl (IS)	1[b]	190	191 (12)	170 (8)
Phenol	0.589	94	136 (14)	66 (12)
o-Cresol	0.698	108	107 (39)	150 (14)
p-Cresol	0.744	208	107 (53)	150 (12)
m-Cresol	0.737	108	107 (44)	150 (15)
Guaiacol	0.876	124	109 (67)	166 (7)
4-Chlorophenol	0.836	128	130 (32)	170 (10)
2,6-Dichlorophenol	0.963	162	164 (64)	204 (14)
2,4-Dichlorophenol	0.992	162	164 (65)	204 (7)
2,4,6-Trichlorophenol	1.116	196	198 (94)	238 (9)
2,4,5-Trichlorophenol	1.188	196	198 (94)	238 (9)
2,3,4,6-Tetrachlorophenol	1.339	232	234 (48)	274 (11)
Pentachlorophenol	1.537	266	268 (63)	308 (13)
6-Chloroguaiacol	1.073	158	160 (33)	200 (6)
4-Chloroguaiacol	1.092	158	160 (32)	200 (5)
5-Chloroguaiacol	1.101	158	160 (32)	200 (9)
4,6-Dichloroguaiacol	1.239	192	194 (62)	234 (4)
3,4-Dichloroguaiacol	1.249	192	194 (63)	234 (5)
4,5-Dichloroguaiacol	1.302	192	194 (62)	234(6)
3,4,6-Trichloroguaiacol	1.357	226	211 (37)	268 (5)
3,4,5-Trichloroguaiacol	1.429	226	228 (96)	268 (6)
4,5,6-Trichloroguaiacol	1.468	226	228 (96)	268 (5)
Tetrachloroguaiacol	1.565	262	264 (47)	304 (5)
3-Chlorosyringol	1.281	188	190 (32)	230 (7)
3,5-Dichlorosyringol	1.364	222	224 (63)	264 (7)
Trichlorosyringol	1.58	256	258 (96)	298 (4)
3-Chlorocatechol	1.186	144	146 (33)	228 (4)
4-Chlorocatechol	1.22	144	146 (32)	228 (3)
3,6-Dichlorocatechol	1.316	178	180 (64)	262 (6)
3,5-Dichlorocatechol	1.332	178	180 (64)	262 (2)
3,4-Dichlorocatechol	1.381	178	180 (63)	262 (4)
4,5-Dichlorocatechol	1.406	178	180 (63)	262 (3)
3,4,6-Trichlorocatechol	1.473	212	214 (94)	296 (5)
3,4,5-Trichlorocatechol	1.539	212	214 (94)	298 (3)
Tetrachlorocatechol	1.659	248	250 (48)	332 (5)
5-Chlorovanillin	1.35	186	188 (33)	187 (25)
6-Chlorovanillin	1.363	186	188 (33)	228 (7)
5,6-Dichlorovanillin	1.512	220	222 (64)	262 (6)
2-Chlorosyringaldehyde	1.524	216	218 (33)	258 (5)
2,6-Dichlorosyringaldehyde	1.674	250	252 (64)	292 (6)
3,5-Dichloro-4-hydroxybenzaldehyde	1.254	189	191 (70)	232 (32)
Surrogate compounds:				
2-Fluorophenol	0.553	112	154 (14)	92 (10)
2-Chlorophenol-d_4	0.781	132	134 (32)	174 (10)
3,4,5-Trichlorophenol	1.268	196	198 (95)	238 (12)
5-Bromo-4-chloroguaiacol	1.421	238	236 (77)	280 (7)
2,4,6-Tribromophenol	1.459	330	332 (94)	372 (8)
Tribromoguaiacol	1.782	360	362 (98)	364 (33)

[a] Mean relative retention time from six point calibration curve.
[b] Mean retention time for internal standard (IS) is 13.17 min (from calibration curve).
[c] Base peak used for quantitation

distilling off the solvent. The reduced volume of solvent was made up to 300 mL with reagent water and acetylated. After completion of survey experiments, the workup for ethanol/toluene Soxhlet extracts was modified to include the addition of 70 mL of reagent water to the extract before the initial concentration step (prior to acetylation).

Sonication extractions

Sonications were performed using a Braun Sonic U sonicator equipped with a 3/4 in. diameter horn. The sample (30 g nominal wet weight) was placed in a 250 mL beaker and 100 mL of solvent added. The mixture was then sonicated (50% duty cycle, 200 W) for 3 min, after which the sample/solvent was transferred to centrifuge tubes and centrifuged for 5 min. The supernatant was collected, concentrated to 10 mL using a Kuderna-Danish (K-D) apparatus, made up to 300 mL with reagent water, and acetylated. The sludge pellet was then re-extracted twice more, and each fraction was collected, acetylated, and analyzed separately. The reported recoveries are the sums of the three partitions.

Soxhlet-Dean-Stark (SDS) extractions

The sample (30 g nominal wet weight) was extracted with toluene (300 mL) for 24 h using a Soxhlet-Dean-Stark apparatus *(5)*. The water from the Dean-Stark trap was collected, made up to 300 mL with reagent water, and acetylated. The toluene from the Dean-Stark trap was collected and added to the toluene in the round bottom flask. A Snyder column was attached to the round bottom flask and the combined toluene extract was concentrated to *ca.* 10 mL using a heating mantle. The concentrated toluene extract was made up to 300 mL with reagent water and acetylated. The reported recoveries are the sums of the respective toluene and water fractions.

Co-extraction steam-distillation (CESD)

Steam distillation followed by a liquid–liquid extraction of the condensed distillate by a small volume of lighter-than-water organic solvent (with the condensed distillate returning to the distillation pot) is termed co-extraction steam-distillation *(6)*. The sample (30 g nominal wet weight) was extracted for 24 h using a co-extraction steam-distillation apparatus with 15 mL of toluene as the extraction solvent. After distillation, the 300 mL of water used in the distillation was filtered through glass wool (to remove the sludge) and acetylated directly. The toluene was collected and diluted to 300 mL with reagent water and acetylated. The reported recoveries are the sums of the respective toluene and water fractions.

Acetylation

The acetylation procedure was adapted from NCASI method CP-86.01 *(7)*. Modifications included the use of a single 300 mL acetylation (instead of 3 x 100 mL) and the removal of the pH adjustment prior to addition of the potassium carbonate buffer (as all extracts were within the pH range 6.5 to 7). Thus, the 300 mL "aqueous" samples were transferred to 500 mL separatory funnels, buffered at pH 11.5 by adding 7.8 mL of 4.34 M K_2CO_3, shaken once or twice, and acetylated by addition of 9 mL re-distilled acetic anhydride. After addition of acetic anhydride, the sample was shaken for *ca.* 30 s (with venting), and then allowed to sit for 5 min. The samples were then extracted with 3 x 40 mL hexane. The three hexane extracts were combined in a 250 mL Kuderna-Danish apparatus and concentrated to *ca.* 5 mL. This volume was then reduced to 1 mL with a gentle stream of nitrogen for those extracts requiring a subsequent cleanup. Those extracts not requiring a cleanup were brought to a final volume of 0.5 mL (using a gentle stream of nitrogen), spiked with 10 µL of 2.5 mg/mL 2,2'-difluorobiphenyl (in hexane) as an injection internal standard, and held for analysis.

Silica gel extract cleanup

Soxhlet extracts of secondary and combined dewatered sludges were subject to cleanup on a silica gel column prior to GC/MS analysis. Cleanup columns (1 cm ID x 30 cm length, with a Teflon stopcock) were prepared by placing a small plug of glass wool in the bottom of the column, adding 5 g activated (135°C for 16 h minimum) silica gel (100–200 mesh), and topping with 1 cm anhydrous sodium sulfate (granular). The columns were pre-eluted with 10 mL hexane and 1 mL of an acetylated extract was added. Each column was then eluted with 10 mL hexane, followed by 120 mL of freshly prepared 35:65 (V:V%) diethylether:hexane. Collection of the eluent was initiated immediately after addition of sample to the column; an initial 15 mL pre-fraction was collected, then a 105 mL analytic fraction. The 105 mL analytic fraction was concentrated to 0.5 mL (KD followed by nitrogen blowdown), spiked with 10 µL of 2.5 mg/mL 2,2'-difluorobiphenyl as an injection internal standard, and held for analysis.

Analysis of extracts

Identification and quantitation of the derivatized phenolics was performed by gas chromatography with mass spectrometric detection(GC/MS, Hewlett Packard 5890/5970 or 5988A). The instrumental conditions employed were as follows: 1.0 µL injections (Grob spitless, purge at 0.8 s); 30 m x 0.25 mm ID x 0.25 µm J & W DB-5 column; initial temp. 50°C, 8°C/min to 270°C, post temp. 300°C for 2 min; injection port at 210°C; GC/MS transfer line at 290°C; helium flow at *ca.* 33 cm/sec.; MS scan range 42–375 AMU. **Table I** lists the target analytes for the method along with their respective quantitations, confirmatory mass fragments, and relative retention times (RRT, relative to 2,2'-difluorobiphenyl); surrogate compounds are also included. For these studies, all quantitations were performed against the injection internal

II. Native concentrations (μg/kg wet weight) recovered from combined dewatered sludge No. 1 with tested extraction techniques

	Soxhlets			Sonication				
	Acetone	Aceto-nitrile	Ethanol/ toluene	Acetone	Aceto-nitrile	Ehanol/ toluene	CESD[a]	SDS[b]
Phenol	59	76	52	81	76	90	58	93
o-Cresol	36	17	307	17	ND[c]	98	87	1540
p-Cresol	35	33	164	ND[c]	184	95	139	835
m-Cresol	104	126	281	53	27	288	280	1113
Guaiacol	923	1220	1220	465	284	371	889	1430
4-Chlorophenol	30	29	28	ND	ND	ND[c]	ND[c]	ND[c]
2,4-Dichlorophenol	22	ND[c]	ND[c]	ND	ND	ND	ND	32
2,4,6-Trichlorophenol	28	27	ND	ND	ND	ND	ND	24
5-Chloroguaiacol	ND[c]	ND	ND	ND	ND	ND	69	ND
4,6-Dichloroguaiacol	22	23	ND	ND	ND	ND	24	17
3,4-Dichloroguaiacol	40	46	45	27	18	ND	37	40
4,5-Dichloroguaiacol	224	311	297	148	81	100	440	251
3,4,5-Trichloroguaiacol	289	349	289	219	130	99	168	212
4,5,6-Trichloroguaiacol	85	96	86	69	46	31	21	64
Tetrachloroguaiacol	43	53	35	ND	ND	ND	43	34
3,5-Dichlorosyringol	ND	52	28	ND	ND	ND	174	ND
Trichlorosyringol	ND	ND	30	ND	ND	ND	ND	ND
3,6-Dichlorocatechol	ND	19	29	ND	ND	ND	ND	ND
3,5-Dichlorocatechol	82	107	126	ND	ND	ND	23	41
4,5-Dichlorocatechol	326	420	594	68	52	41	19	162
3,4,6-Trichlorocatechol	ND	25	65	ND	ND	ND	ND	ND
3,4,5-Trichlorocatechol	352	761	1830	ND	24	ND	40	178
Tetrachlorocatechol	87	275	408	ND	ND	ND	ND	26
6-Chlorovanillin	ND	ND	40	ND	ND	ND	163	ND
5,6-Dichlorovanillin	ND	ND	30	ND	ND	ND	42	ND
2,6-Dichlorosyringaldehyde	ND	ND	33	ND	ND	ND	24	ND

[a] Co-extraction steam-distillation.
[b] Soxhlet Dean-Stark.
[c] Not detected at 16 μg/kg.

standard (2,2'-difluorobiphe-nyl) using average response factors generated from a six-point calibration curve spanning the range 1.5 to 50 μg/mL (final extract concentration). The calibration standards were prepared by spiking the appropriate levels of unacetylated standards into 300 mL reagent water and acetylating (without cleanup).

Results and discussion

Method development was approached as a two-stage process. The first phase was a comparison of different extraction techniques to determine the optimum technique for extracting phenolics (from a combined dewatered sludge); the second phase was optimization and characterization of the final method.

Results of studies performed by Remberger *et al. (1)* clearly demonstrate that polar and/or water miscible solvents are required to effectively extract phenolics from sediments. However, for the "traps" to function effectively, CESD and SDS require nonmiscible, lighter than water solvents. Thus, in these cases, the range of suitable solvents is limited.

Considering that NCASI has extensive experience with the aqueous *in situ* acetylation, this procedure was adapted for use in the reported solids method. By diluting the reduced volume of each organic extract into a large volume of water the potential for matrix-effects on the acetylation due to the different solvents was minimized. However, all the "aqueous" samples held residual solvent when acetylated. Acetylation tests performed with acetone, acetonitrile, ethanol/toluene, and acetone/hexane showed that the acetylation was not adversely affected by the presence of 5% of these solvents (15 mL in 300 mL). Although

III. Ranking of native concentrations found by Soxhlet extraction of combined dewatered sludge No. 1 using various solvents; on a scale of 1 to 8 (eight solvents), the highest concentration is ranked 1

	Acetone	*Aceto-nitrile*	*Acetone/ hexane*	*Ethanol/ toluene*	*Methylene chloride*	*Acetic acid/ hexane*	*sopropanol/ hexane*	*Acetonitrile/ methanol*
Phenol	2	1	7	4	6	5	3	8
m-Cresol	3	2	8	1	7	5	6	4
Guaiacol	5	1	6	2	8	3	7	4
3,4-Dichloroguaiacol	3	1	8	2	6	4	5	7
4,5-Dichloroguaiacol	4	1	7	2	8	3	5	6
3,4,5-Trichloroguaiacol	3	1	8	2	7	5	4	6
Tetrachloroguaiacol	2	1	8	5	6	3	4	7
3,5-Dichlorocatechol	6	4	3	1	8	5	2	7
4,5-Dichlorocatechol	5	4	2	1	8	7	3	6
3,4,5-Trichlorocatechol	7	4	2	1	8	5	3	6
Tetrachloroca'echol	6	3	4	1	8	7	2	5
Scores	49	24	68	24	87	56	50	74

IV. Ranking of scores from Soxhlet extraction of different matrices using different solvents

Matrix	*Acetone*	*Aceto-nitrile*	*Ethanol/ toluene*	*Isopropanol/ hexane*
Combined dewatered sludge No. 1	2	1.5[a]	1.5[a]	3
Pulp No. 1	2	1[a]	4	3
Pulp No. 2	3	1	2	4[b]
Pulp No. 3	3[b]	2	1[a]	NT[c]
Secondary sludge No. 1	NT[c]	1.5	1.5	2

[a]Significantly low score (95% confidence level)
[b]Significantly high score (95% confidence level)
[c]Not tested

the efficiency of the aqueous acetylation was not adversely affected by small amounts of solvent, difficulties associated with quantitatively removing the organics prior to acetylation resulted in acetylations being performed with up to 20% organic present. Thus, results reflect not only the extractive ability of the tested extraction procedures, but also any effect of the residual organic on the acetylation and subsequent hexane extraction. For the purposes of this study, these (potential) effects were considered inseparable from the respective extraction method itself.

Comparison of extraction techniques

Survey experiments to screen extraction techniques were performed using a combined dewatered sludge (CDWS). This matrix contained components of primary as well as secondary sludge and was thus considered to be an ideal test case. In order to obtain the most accurate measure of the relative extractive ability of the techniques tested, comparisons were based solely on the recovery of native species. This approach removes uncertainties associated with interpreting recoveries from fortified samples *(1)*, i.e., variability related to spiking methodology (mixing etc.) and equilibration time (free vs. bound, etc.).

Table II lists the native analyte concentrations (µg/kg wet weight) obtained using the various extraction methods tested. Some general trends are apparent in these data. Of the tested procedures, SDS recovers the highest levels of nonchlorinated phenolics; Soxhlet extraction, CESD, and SDS recover approximately the same levels of guaiacols; CESD recovers the highest levels of vanillins and syringols; and Soxhlet extraction recovers the highest levels of catechols. The low frequency of detects limits any substantive discussion for phenols themselves. Overall, sonication performs quite poorly relative to the other procedures tested.

Although CESD gives marginally greater recoveries of select chloroguaiacols, the tested methods generally perform equally well for the chloroguaiacols as a group (with the exception of sonication). On the other hand, Soxhlet extraction recovers concentrations for some catechols up to an order of magnitude greater than CESD or SDS. In addition, ethanol/toluene Soxhlet extraction recovers approximately the same levels of vanillins, syringols, and syringaldehydes (with the exceptions of 6-chlorovanillin and 3,5-dichlorosyringol)

as CESD. Thus, overall, these data indicate Soxhlet extraction is the most effective "nonaggressive" technique for recovering the widest range of compounds of interest, and further studies focussed on finding the optimum solvent for use with Soxhlet extraction.

Comparisons of solvents for Soxhlet extraction

The same combined dewatered sludge was Soxhlet extracted using eight different solvents. In order to simplify interpretation of the resulting data, the quantitations from each solvent for each analyte were ranked on a scale of 1 to 8 (eight solvents), with the solvent recovering the highest level of that specific compound given a score of 1. Only the 12 compounds found in all replicates were included. Thus, if one solvent gave the greatest recovery for each of the 12 species, a score of 12 would result. The appropriate rankings and scores (the summed ranks) are given in **Table III**. As presented, this type of ranking test is similar to that recommended for interlaboratory comparisons by AOAC *(8)*. For eight solvents and 12 analytes, the upper and lower limits defining significant bias at the 95% confidence level are 76 and 32, respectively *(8)*. Thus, by this analysis, both acetonitrile and ethanol/toluene are significantly better solvents than the others tested; by the same analysis, only methylene chloride can be rejected. To expedite further comparisons, solvents scoring above the mean score of 54 were not carried further. Thus, acetone, acetonitrile, ethanol/toluene, and isopropanol/hexane were tested with three bleached pulps and a secondary sludge.

Table IV lists the ranked scores for each matrix tested with the four solvents scoring above 54 in the initial test; for a specific matrix, the solvent giving the lowest score (analogous to the scores listed in Table III) receives a rank of 1 (ties result in fractional ranking). The experiments were performed in the order listed in Table IV. Isopropanol/hexane was not tested with pulp No. 3 because it would not cycle effectively with pulp No. 2 due to the relatively high water content of this sample (*ca.* 7% solids); it was included in the tests with secondary sludge No. 1 in order to maintain a minimum of three solvents per test. Due to the low levels of native analytes, the analysis of the pulp samples (only) were performed using SIM GC/MS; no compounds were detected in bleached pulps at levels greater than 50 µg/kg (wet weight).

Overall, the data from the five test matrices show that acetonitrile and ethanol/toluene are the most effective solvents for use with Soxhlet extraction of the tested matrices. Generally, the factor differentiating the solvents is catechol recoveries; depending on the matrix, recoveries for catechols with ethanol/toluene can be twice those obtained with acetonitrile. On the other hand, ethanol/toluene consistently gave concentrations within 20% of those obtained with acetonitrile. The decision to use ethanol/toluene was also influenced by the additional practical consideration of boiling points, which determine the ease of conversion to an aqueous phase prior to acetylation. The ethanol/toluene/water and ethanol/water (water from the sample itself) azeotropes boil at *ca.* 74°C and 77°C, respectively, and the ethanol/toluene extract can be concentrated to a small volume of essentially 100% water or ethanol (depending on the amount of water extracted from the sample) with a water bath; acetonitrile boils at *ca.* 82°C and is difficult to concentrate in a water bath (thus making it difficult to obtain a reasonable volume of a predominantly aqueous phase for acetylation).

With the decision to use ethanol/toluene as the extraction solvent, the extract workup was modified to ensure a 100% aqueous phase for acetylation. This was accomplished by adding 70 mL reagent water to the ethanol/toluene extract prior to the initial concentration step. Thus, sufficient water is present to ensure that essentially 100% of the organics are distilled off during the Kuderna-Danish concentration (first as the ethanol/toluene/water ternary azeotrope, and then as the ethanol/water azeotrope), leaving a minimum of 50 mL water. An additional benefit of this approach is that the extract cannot be taken to dryness during the concentration step because the final aqueous phase will not distill at water bath temperatures (*ca.* 95°C). This modification was tested, and it was found that losses due to this specific step in the extract workup were less than 10%.

Silica gel clean-up

Initially, it was hoped that the hexane extraction performed after the aqueous *in situ* acetylation would be sufficient as a cleanup. During the early phases of the extraction comparison tests, it became evident that for Soxhlet extracts of combined dewatered and secondary sludges this was not the case. With these specific extracts, injection of a single extract could result in deterioration of the chromatographic system such that removal of the first few centimeters of the column and replacement of the injection port liner were necessary. It is important to note that extracts obtained by CESD, SDS, or sonication did not have this effect, nor did Soxhlet extracts from pulps. These observations indicate firstly that CESD, SDS, and sonication are not as aggressive as Soxhlet extraction, and secondly, that bleached pulps are essentially a "clean" matrix.

Once it became apparent that a cleanup was necessary for some extracts, a silica gel column cleanup was developed. The absolute recoveries from the silica gel column have been characterized, and recoveries ranged from a low of 70% (tetrachlorocatechol and 2,6-dichlorosyringaldehyde) to a high of 91%.

For this work, all extracts of combined dewatered and secondary sludges were run through a silica column prior to GC/MS analysis. While this manuscript was in preparation, it was

V. Percent recoveries from Soxhlet extractions of fortified (matrix spike and extract spike) secondary sludge No. 1 (n=2); spike level ≥ three times native concentrations for all species

	Extract spikes, Mean (S.D.)[a]	*Matrix spikes, Mean (S.D.)*	*Relative matrix spike recoveries, Mean (S.D.)*
Phenol	65 (12)	75 (7)	115 (24)
o-Cresol	184 (11)	269 (31)	146 (19)
p-Cresol	177 (10)	292 (53)	165 (32)
m-Cresol	97 (4)	130 (1)	134 (5)
Guaiacol	80 (4)	92 (6)	114 (9)
4-Chlorophenol	85 (3)	80 (5)	94 (7)
2,6-Dichlorophenol	87 (4)	81 (6)	92 (9)
2,4-Dichlorophenol	88 (6)	83 (7)	93 (10)
2,4,6-Trichlorophenol	95 (5)	87 (5)	92 (7)
2,4,5-Trichlorophenol	97 (5)	85 (5)	88 (7)
2,3,4,6-Tetrachlorophenol	105 (2)	94 (1)	89 (2)
Pentachlorophenol	113 (5)	99 (1)	88 (4)
6-Chloroguaiacol	92 (4)	84 (5)	91 (7)
4-Chloroguaiacol	91 (5)	84 (5)	92 (8)
5-Chloroguaiacol	94 (5)	85 (6)	90 (8)
4,6-Dichloroguaiacol	100 (4)	89 (5)	89 (6)
3,4-Dichloroguaiacol	101 (3)	91 (4)	91 (5)
4,5-Dichloroguaiacol	104 (3)	94 (3)	91 (4)
3,4,6-Trichloroguaiacol	102 (4)	93 (2)	91 (4)
3,4,5-Trichloroguaiacol	115 (15)	86 (4)	75 (10)
4,5,6-Trichloroguaiacol	106 (3)	95 (1)	90 (3)
Tetrachloroguaiacol	125 (13)	103 (2)	82 (9)
3-Chlorosyringol	99 (1)	90 (3)	91 (4)
3,5-Dichlorosyringol	103 (3)	91 (2)	89 (3)
Trichlorosyringol	117 (4)	107 (1)	92 (3)
3-Chlorocatechol	69 (2)	40 (6)	58 (9)
4-Chlorocatechol	69 (4)	57 (9)	82 (14)
3,6-Dichlorocatechol	80 (3)	13 (1)	16 (2)
3,5-Dichlorocatechol	83 (5)	35 (0)	43 (3)
3,4-Dichlorocatechol	70 (5)	27 (3)	39 (5)
4,5-Dichlorocatechol	98 (12)	67 (3)	68 (9)
3,4,6-Trichlorocatechol	76 (5)	12 (1)	15 (2)
3,43,5-Trichlorocatechol	124 (19)	67 (15)	54 (14)
Tetrachlorocatechol	116 (14)	36 (8)	31 (8)
5-Chlorovanillin	95 (2)	81 (2)	85 (3)
6-Chlorovanillin	104 (2)	92 (1)	88 (1)
5,6-Dichlorovanillin	109 (3)	94 (2)	86 (3)
2-Chlorosyringaldehyde	109 (4)	102 (3)	94 (5)
2,6-Dichlorosyringaldehyde	116 (3)	110 (9)	95 (8)
3,5-Dichloro-4-hydroxbenzaldehyde	106 (5)	89 (2)	84 (4)

[a]Standard deviation

observed that the silica gel cleanup was not effective with a specific combined dewatered sludge. After analysis of two extracts of this specific sample, the GC column required an extended bake-out in order to obtain a "clean" blank chromatogram (without changing the injection port liner). Even with frequent bake-outs, a cumulative effect on recoveries was observed after multiple sample analyses.

Characterization of overall extraction and workup efficiency

As a means of characterizing the efficiency of the extract workup, unacetylated standards were spiked into Soxhlet extracts prior to the initial concentration step ("extract spikes"). The recoveries of these added standards give a direct measure of the losses associated with sample workup. In addition, "true" matrix spike experiments (unacetylated standards added to the sludge prior to extraction) were performed simultaneously. Note that these compounds were added to the sample immediately prior to extraction (i.e., no extended equilibration with the sample prior to extraction).

Table V lists the percent recoveries from extract and matrix spike experiments performed with a secondary sludge. From Table V, the extract spike recoveries range from 65% for phenol to 184% for *o*-cresol. Overall, these recoveries show that losses from the extract workup (including cleanup) are consistently less than 30%. Considering that fully 20% of this loss can be reasonably accounted for by the two KD concentration steps (which the calibration standards do not undergo), this was judged acceptable.

By calculating relative percent recoveries for the matrix spike experiments using the recoveries from the extract spike, the extraction itself can be isolated from the rest of the workup and a measure of extraction efficiency, and thus an estimate of method accuracy, was obtained. These relative percent recoveries for matrix spike experiments are also given in Table V.

From these data, ethanol/toluene Soxhlet extraction recovers 80% or better of all the spiked compounds from this matrix except the chlorocatechols. An identical set of experiments was also performed with a combined dewatered sludge with analogous results.

The contrast between the matrix spike recoveries of chlorocatechols and all other analytes suggests that catechols "bind" to the solids via a different mechanism than the other compounds. Remberger *et al. (1)* have postulated that catechols might bind via formation of bidentate ligand complexes with species such as Fe(III). In addition, the same workers have presented data indicating that catechols can become "bound" to solids such that they cannot be recovered by "nonaggressive" techniques in as little as 15 min *(9)*. Thus, the low matrix spike recoveries of the chlorocatechols may reflect rapid binding, and not poor recovery of "free" moieties. Indeed, the fact that the solvent extraction does not recover a fraction of the spiked compounds means this component is "bound" by definition. However, these characterizations are open to debate due to the operational nature of the definitions.

Overall, the assessment of accuracy in trace environmental analyses is difficult at best because it is often impossible to know what the "true" value is for a given sample. A common means of estimating accuracy is the use of matrix spike recoveries, as presented above. However, the spiking procedure in no way can be assumed to mimic the equilibrated native species, and thus matrix spike recoveries represent a very optimistic estimate of accuracy *(1)*.

VI. Results (µg/kg wet weight) from replicate (n=7) analyses by ethanol/toluene Soxhlet extraction

	Combined dewatered sludge No. 2		*Combined dewatered sludge No. 3*		*Secondary sludge No. 2*	
	Mean	*% RSD*	*Mean*	*% RSD*	*Mean*	*% RSD*
Phenol	500	10	ND	[b]	ND	[c]
o-Cresol	1700	[a]	1600	[a]	690	22
p-Cresol	1300	[a]	1100	[a]	414	23
m-Cresol	1300	[a]	889	21	314	21
Guaiacol	63	18	132	27	ND	
2,6-Dichlorophenol	17	8	ND		ND	
2,4-Dichlorophenol	50	8	29	10	ND	
2,4,6-Trichlorophenol	98	6	232	9	ND	
2,3,4,6-Tetrachlorophenol	36	10	70	8	ND	
Pentachlorophenol	41	12	41	7	ND	
4,6-Dichloroguaiacol	ND	[b]	23	7	ND	
3,4-Dichloroguaiacol	27	10	65	7	ND	
4,5-Dichloroguaiacol	60	17	484	12	ND	
3,4,6-Trichloroguaiacol	39	14	72	7	ND	
3,4,5-Trichloroguaiacol	918	9	430	9	244	11
4,5,6-Trichloroguaiacol	36	11	216	6	ND	
Tetrachloroguaiacol	772	11	227	7	211	12
3,5-Dichlorosyringol	ND	34	16		ND	
Trichlorosyringol	139	10	111	8	61	9
3,6-Dichlorocatechol	18	47	ND		ND	
3,5-Dichlorocatechol	23	31	32	35	ND	
3,4-Dichlorocatechol	17	27	ND		ND	
4,5-Dichlorocatechol	198	16	116	32	160	13
3,4,5-Trichlorocatechol	195	31	260	29	497	32
Tetrachlorocatechol	92	22	110	13	465	41
6-Chlorovanillin	48	20	272	15	ND	
5,6-Dichlorovanillin	19	18	85	10	ND	
2-Chlorosyringaldehyde	ND		45	12	ND	
2,6-Dichlorosyringaldehyde	ND		83	13	ND	
3,5-Dichloro-4-hydroxybenzaldehyde	17	28	ND	ND	ND	

[a]Outside calibration range
[b]Not detected at 16 µg/kg (ppb)
[c]Not detected at 50 µg/kg (ppb)

Single laboratory precision for ethanol/toluene Soxhlet extraction

The calibration limits selected for the GC/MS analysis were based on prior experience with chlorophenolics in wastewater analyses. The low end of the calibration range was selected based on an estimate of the minimum quantity required for spectral identification [*ca.* 1.5 ng (nanogram) on column]; note that this is an estimate only and the true minimum quantity identifiable will be dependent on the specific species considered, the condition of the instrumentation, and the level of interfering compounds. The high end of the calibration range was selected based on an estimate of the maximum amount which can be placed onto the column without observing column overload (*ca.* 50 ng on column). Thus, the calibration spans the range from 25 to 833 µg/kg (30 g sample, wet weight basis). Given the wide range in sample consistencies (*ca.* 5 to 50% solids), these limits correspond to 500 µg/kg to 17 mg/kg (30 g sample, 5% solids, dry weight basis), or 50 µg/kg to 1.7 mg/kg (30 g sample, 50% solids, dry weight).

VII. Mean recoveries of surrogate compounds from precision replicates (by matrix type)

	CDWS[a] *% Rec. (S.D)*	*Secondary sludge*[b] *% Rec. (S.D.)*
3,4,5-Trichlorophenol	79 (5)	83 (7)
2-Chlorophenol-d_4	75 (7)	68 (6)
5-Bromo-4-chloroguaiacol	97 (6)	110 (10)
2-Fluorophenol	66 (10)	65 (9)
2,4,6-Tribromophenol	103 (7)	112 (15)
Tribromoguaiacol	65 (13)	113 (14)

[a]Combined dewatered sludge, n=14
[b]n=7

VIII. Initial concentrations (µg/kg wet weight) found in combined dewatered sludge No. 2, and mean relative percent differences (RPD) in concentrations found after storage at -15°C for various times

	Day 0 (n=7)		*Day 20 (n=3)*	*Day 37 (n=3)*	*Day 65 (n=3)*	*Day 156 (n=3)*
	Mean	*RSD*	*RPD*	*RPD*	*RPD*	*RPD*
Phenol	500 µg/kg	10	4	19	-1	12
o-Cresol	1700[a]	19	-37	-11	-28	-6
p-Cresol	1300[a]	20	-36	-13	-27	0
m-Cresol	1399[a]	16	-44	-15	-27	-5
Guaiacol	63	18	-11	5	9	27
2,6-Dichlorophenol	17	8	-8	35	-16	ND[c]
2,4-Dichlorophenol	50	8	-9	12	-8	4
2,4,6-Trichlorophenol	98	6	-3	-5	-13	8
2,3,4,6-Tetrachlorophenol	36	10	-6	-8	-18	-11
Pentachlorophenol	41	12	17	10	-4	-21
3,4-Dichloroguaiacol	27	10	-10	7	-7	-33[b]
4,5-Dichloroguaiacol	60	17	-22	-3	-15	3
3,4,6-Trichloroguaiacol	39	14	20	26	10	-11
3,4,5-Trichloroguaiacol	918	9	10	12	-17	-31[b]
3,4,6-Trichloroguaiacol	36	11	14	24	-3	-10
Tetrachloroguaiacol	772	11	7	10	-19	-33[b]
Trichlorosyringol	139	10	5	13	-17	-38[b]
3,6-Dichlorocatechol	18	47	ND[c]	ND[c]	ND[c]	ND
3,5-Dichlorocatechol	23	31	25	12	ND	ND
3,4-Dichlorocatechol	17	27	62	-10	74	110
4,5-Dichlorocatechol	198	16	12	33	33	72[b]
3,4,5-Trichlorocatechol	195	31	8	28	39	147
Trichlorocatechol	92	22	74	49	20	38
6-Chlorovanillin	48	20	-21	0	-5	-4
5,6-Dichlorovanillin	19	18	-18	0	-7	32
3,5-Dichloro-4-hydroxybenzaldehyde	17	28	32	-1	40	41

[a]Outside calibration range.
[b]Difference is significant at 95% confidence level (1-tailed pooled t-test)
[c]Not detected at 16 µg/kg.

The overall precision of the procedure was assessed by analyzing seven replicates (each) of two combined dewatered sludges and a single secondary sludge. The results from these analyses are given in **Table VI**, and reflect the ability to identify 1 ng injections. In all cases, the results represent native analytes present in the sample and, thus, provide a realistic estimate of the reproducibility of the method.

QA/QC considerations

Throughout the process of method development, a group of potential surrogate compounds were carried through all experiments. As part of the precision study 3,4,5-trichlorophenol, 2-chlorophenol-d_4, and 5-bromo-4-chloroguaiacol were spiked into/onto the samples prior to extraction (extraction surrogates), and 2-fluorophenol, 2,4,6-tribromophenol, and tribromoguaiacol (uncharacterized isomer) were spiked into the extract prior to the initial concentration step (workup surrogates). The goal of this spiking scheme was to differentiate poor extraction efficiencies from matrix effects in the workup. In addition, the recoveries of specific workup surrogates are indicative of acetylation and cleanup efficiency. 2,4,6-Tribromophenol is an early eluter in the silica cleanup, and low recoveries of this species would indicate a problem with the cleanup (note, 3,4,5-trichlorophenol recovery should then also be low). Tribromoguaiacol is an indicator of acetylation efficiency, and 2-fluorophenol is an indicator of volatilization loss from the concentration steps.

Table VII lists the mean surrogate recoveries obtained from the precision study (Table VI). Although these data represent recoveries from a very limited number of matrices, they represent achievable method performance.

Storage stability

An important feature of secondary sludges (and therefore combined dewatered sludges to a lesser degree), ASB sediments, and river sediments is the presence of microbial organisms. Reports in the literature clearly indicate a potential for biotransformation *(9, 10)* of the target analytes within these matrices and therefore, an effective means of suppressing biological activity for sample storage was needed. Although pH adjustment is used to stabilize bio-activity in wastewater analyses for chlorinated phenolics *(3)*, this is not a practical approach for pulps or sludges due to variability in moisture content and buffer capacity, as well as difficulties associated with ensuring a homogeneous pH in the sample after addition of reagents. The most practical approach seemed to be freezing, and studies were undertaken to determine if this was an effective means of fixing analyte concentrations.

To assess the utility of freezing as a mode of storage, large quantities of a secondary sludge (after centrifuging) and a combined dewatered sludge were composited, partitioned, and frozen (–15°C). Initially, seven replicate analyses (Table VI) were performed on each sample. At approximately weekly intervals, an aliquot of each sample was thawed, and triplicate reanalyses performed.

Table VIII summarizes the results from the reanalyses of CDWS No. 2. From these data, a trend toward decreasing guaiacol levels begins to appear at 65 days, and the concentrations of 3,4-dichloroguaiacol, 3,4,5-trichloroguaiacol, tetrachloroguaiacol, and trichlorosyringol have decreased significantly by 156 days. Over this period of time, catechol recoveries appear to be increasing; however, the poorer precision for the catechol measurements results in only 4,5-dichlorocatechol showing a statistically significant increase. The results from the reanalyses of the secondary sludge showed no significant changes in concentrations up to five months. Overall, these data suggest a five-month maximum storage time at –15°C.

Conclusions

Of the methods and solvents tested, Soxhlet extraction with ethanol/toluene gives the greatest recoveries of native concentrations for the widest range of compounds examined. By definition *(1)*, species recovered by "direct solvent extraction" are "free." Thus, the reported method allows quantitation of "free" species at levels below 100 µg/kg with good precision (20–30%RSD). Results from the storage stability study showed significant changes after *ca.* five months at –15°C, and samples should be analyzed within this time period. ■

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