Journal of Chromatography A, 662 (1994) 281–292
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 679

Indentification and quantification of mono-, di- and trihydroxybenzenes (phenols) at trace concentrations in seawater by aqueous acetylation and gas chromatographic–mass spectrometric analysis

Thomas J. Boyd*

0218, Marine Life Research Group, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0218 (USA)

(First received August 2nd, 1993; revised manuscript received October 22nd, 1993)

ABSTRACT

A method is described that uses aqueous acetylation with acetic anhydride, solid-phase extraction (SPE) and concentration, and gas chromatographic–mass spectrometric (GC–MS) analysis to identify and quantify mono-, di- and trihydroxybenzenes (phenols) at low concentrations in marine and waste waters. Phenolic compounds in water samples were buffered with $NaHCO_3$ and directly acetylated with acetic anhydride. Phenol acetates were then extracted using C_{18} SPE columns. The columns were eluted and the phenol acetates concentrated and analyzed by GC–MS. Detection of phenols in the ng l^{-1} concentration range can be obtained with 500 ml of sample. A large volume extraction setup is described from which detection limits in the upper pg l^{-1} range may be obtained. The method was applicable to a wide range of phenolic compounds but was not suitable for nitrophenols. Water samples from several coastal environments and wastewater treatment facilities were analyzed for phenols using this method. Phenol, cresols and catechols were the most common phenolic compounds identified. Concentrations ranged from 2.5 to 370 ng l^{-1} for these phenols in seawater sampled in San Diego Bay, in the vicinity of White's Point outfall off San Pedro (Los Angeles area) and outfalls off the Northern coast of California near Eureka.

INTRODUCTION

Phenolic materials are present in many environments. Their ubiquitous presence in industrial and municipal wastewaters, groundwaters, sediments, and soils [1–9] has made them of interest to chemists, waste managers, and public agencies such as the United States Environmental Protection Agency (EPA). Phenolic materials are also present in natural samples and have been identified in such environments as streams, lakes [10,11], estuaries [7,12], freshwater and marine sediments [13–17] and marine microlayers [18,19]. Phenolic moieties are also found in marine algae [20–22] and in humic materials from terrestrial sources [23,24].

Many analytical approaches have been used for the identification and quantification of phenolic materials under various environmental conditions. The EPA has approved several analytical methods for the analyses of these materials [25,26]. In addition, there is considerable interest from researchers and managers to devise new, definitive methods for phenolic analysis [27]. Most commonly, phenols are extracted from water samples by liquid extraction and quantified by gas chromatography (GC) or liquid chromatography (LC) [28–30]. Several colorimetric analyses also exist. Generally, most involve derivatizing the phenolic moiety with a

* Present address: Ocean Sciences Division, Room 725, National Science Foundation, 4201 Wilson Boulevard, Arlington, VA 22230, USA.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1125-J

chromophoric substance for bulk colorimetric or LC separation and determination [31–33].

Derivatization of phenols, besides the potential to introduce chromophores, may also be used to introduce a halogenated group in the native molecule. Derivatized phenols thus become analyzable by extremely sensitive GC with electron-capture detection (ECD) [34,35]. Use of a derivatizer, such as a silanizing agent, makes phenols less polar, greatly increasing extraction efficiency [27,29]. Alteration of the phenolic molecule by acetylation may optimize GC analysis for a wide range of phenols on standard non-polar columns by affecting retention characteristics.

Recently, acetic anhydride has been used as a derivatizing agent for extracted phenols [30,34,36] and also for aqueous solutions [1,37,38]. Use of excess buffer and controlled conditions are essential for successful acetylation. Once acetylated, however, extraction efficiency of phenols is increased substantially. Use of acetic anhydride has several advantages that are exploited in this study: (1) addition of the acetyl group decreases the polarity of the phenolic analyte allowing for greater extraction efficiency [37], especially from di- and trihydroxybenzenes which are generally not extractable from aqueous samples by standard methods, (2) low cost of buffer salts and acetic anhydride makes the derivatization of large water samples economically feasible, (3) phenolic acetate formed by derivatization has very similar mass spectral characteristics to the original underivatized phenol, resulting in more successful spectral library searches, and (4) a characteristic peak 42 mass units higher than the parent phenol molecular ion peak may be used to identify an acetylated substance thus serving as a qualitative indicator of the phenolic moiety. Fragments showing a characteristic loss of ketene $M^+ - 42$, also may confirm the molecule as a phenol acetate.

Phenols in the marine environment are less well characterized than their terrestrial counterparts. Phenolic materials from terrestrial sources entering the ocean are subject to considerable physical, chemical and biological influence. Rapid dilution [39], photochemical bleaching [40–42], and possibly biodegradation [43–45] may lead to extremely low concentrations in coastal environments. The need to identify and quantitate phenolic and other toxic materials in marine waters has been set as a national objective and the development of new and improved techniques for its accomplishment are called for in the Federal Plan for Ocean Pollution Research, Development, and Monitoring [46]. This study was undertaken to develop a relatively rapid, accurate and reliable method for the determination of phenolic materials at extremely low concentrations in coastal marine environments.

EXPERIMENTAL

Chromatographic apparatus and scintillation counter

The gas chromatograph–mass spectrometer was a Hewlett-Packard 5988 Chemstation with a 7673A autosampler. The HPLC apparatus used to purify [2,6-^{3}H]*p*-cresol consisted of a Perkin-Elmer Series 410 LC pump, Wescan Model 272 UV absorbance detector (Japan Spectroscopic Co.), and a Hewlett-Packard Model 3390A integrator. The scintillation counter used in the purification of [2,6-^{3}H]*p*-cresol was a Beckman Model LS1801 (Beckman Instruments, Irvine, CA, USA).

Chromatographic conditions

A glass capillary AT-35 (65% methyl/35% phenyl) column, 25 m × 0.25 mm I.D. (Alltech, Deerfield, IL, USA) was used for all samples. A sample volume of 3 μl was used for each injection. The operating conditions were: column temperature: 40°C, hold 6 min; 40 to 275°C at 5°C min^{-1}, hold 5 min; injector port temperature, 190°C; linear velocity: 40 cm s^{-1} (set at 150°C), helium; mass spectrum with electron impact source tuned with perfluorotributylamine (PFTBA); source temperature, 180°C; scan 50–500 u with 100 abundance threshold; splitless injection. A 1 g l^{-1} solution of butylated hydroxytoluene was used as an external standard for day-to-day calibration of the instrument.

For HPLC purification of [2,6-^{3}H]*p*-cresol, a Waters Nova-Pak C_{18}, 4 μm particles, 150 mm × 3.9 mm steel column was used. The operating conditions were: 0 min, water (1% acetic acid)–

MeOH (1% acetic acid) (95:5) hold 2 min; 2–5 min ramp to water–MeOH (65:35), hold 10 min; detector set to 280 nm absorbance. Purified [2,6-^{3}H]*p*-cresol was analyzed in the scintillation counter and the specific activity (11 Ci mmol^{-1}) was calculated.

Large-volume extraction apparatus

For sample volumes over 1 l, a large-volume extraction apparatus consisting of the following components was used: A 19-l stainless-steel sample container (Cornelius, Anoka, MN, USA) fitted with PTFE O-rings and seals and stainless-steel sparger element, was employed to hold water samples. The container was sparged with nitrogen gas run through a Hewlett-Packard Model 19046A gas purifier within 1 h of sample collection. Containers were sparged soon after collection in an effort to inhibit aerobic microbial activity, particularly utilization of phenolic analytes. In addition, $NaHCO_3$ was also added soon after collection in an effort to alter the collected sample environment. The extra sodium load (0.35 *M* addition) was assumed to inhibit normal microbial activity. A PTFE hose connected to the stainless-steel container carried water flow through a 147 mm 0.3 μm precombusted glass fiber filter (Gelman Sciences, Ann Arbor, MI, USA) in a Millipore filter holder (Millipore, Bedford, MA, USA). Flow continued through a PTFE hose to a PTFE manifold and hose assembly and then into four pre-packed C_{18} solid-phase extraction (SPE) columns (see *Preparation of SPE columns* below). The columns were secured to a vacuum manifold through which extracted water flowed into a large (19 l) carboy trap (Corning, East Brunswick, NJ, USA).

Preparation of SPE columns

For sample volumes of 1 l or less, commercially available Prep-Sep C_{18} SPE columns (Fisher Scientific, Tustin, CA, USA) were used for extraction. For larger sample volumes, columns were constructed from 60-ml polypropylene syringes. Each syringe was fitted with a 20-μm pore frit (Varian, Harbor City, CA, USA) and loaded with 5 g of 40 μm C_{18} sorbent (Analytichem, Harbor City, CA, USA). The sorbent was capped with another frit. Columns were soaked for several minutes with Optima-grade methanol, vacuumed dry and immediately soaked with HPLC grade water prior to use.

Reagents

NaCl (pre-combusted at 550°C) was used to prepare 3 *M* artificial seawater (ASW). ASW was prepared freshly prior to use in preparations of standards. Water used in the preparation of all standards was HPLC grade from a Milli-Q purification apparatus (Millipore). Acetic anhydride, methanol, 2,7-dihydroxynaphthalene, $NaHCO_3$ and anhydrous Na_2SO_4 were of HPLC grade and purchased from Fisher Scientific. Butylated hydroxytoluene was purchased from Sigma (St. Louis, MO, USA). Na_2SO_4, used for drying organic extracts was pre-baked at 550°C. $NaHCO_3$ was cleaned by extraction with 35 volumes of CH_2Cl_2 (Fisher Scientific) in a Soxhlet extractor prior to use. Scintillation cocktail used was Ecoscint obtained from National Diagnostics, Manville, NJ, USA.

Preparation of standard solutions

Stock solutions of 1 g l^{-1} phenol (Mallincrodt, St. Louis, MO, USA), *o*-cresol, *m*-cresol, catechol, phloroglucinol (Sigma), *p*-cresol, 3-methylcatechol and 4-methylcatechol (Phaltz & Bauer, Waterbury, CT, USA) were prepared in methanol. A commercial standard containing 2,4,6-trichlorophenol, 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and pentachlorophenol at concentrations of 0.5–2.5 g l^{-1} (Supelco, Bellefonte, PA, USA) was also used. These stock solutions were diluted in ASW to give the final standard concentrations listed in the following sections.

Preparation of standard test solutions

Two series of phenolic standard solutions were prepared as follows: one, in which the sample volume was kept constant and concentration was varied, and one, in which sample concentration was kept constant and the sample volume was varied. For constant-volume (0.50 l) standards, test solutions were made using ASW and phenol stock solutions to concentrations of 0.500, 1.00, 2.50, 5.00 and 10.0 μg l^{-1} for *o*-cresol, *m*-cresol, *p*-cresol, catechol, 3-methylcatechol, 4-methylcatechol and phloroglucinol; 0.250, 0.500, 1.25,

2.50 and 5.00 $\mu g\ l^{-1}$ for phenol, 2-chlorophenol, 2-nitrophenol, 2,4-dimethylphenol and 2,4-dichlorophenol; 1.25, 2.50, 6.25, 12.5 and 25.0 $\mu g\ l^{-1}$ for 4-chloro-3-methlyphenol, 4-nitrophenol and pentachlorophenol; and 0.750, 1.50, 3.75, 7.50 and 15.0 $\mu g\ l^{-1}$ for 2,4,6-trichlorophenol and 2,4-dinitrophenol.

For constant-concentration standards at 1.00 $\mu g\ l^{-1}$ for *o*-cresol, *m*-cresol, *p*-cresol, catechol, 3-methylcatechol, 4-methylcatechol and phloroglucinol; 0.500 $\mu g\ l^{-1}$ for phenol, 2-chlorophenol, 2-nitrophenol, 2,4-dimethylphenol and 2,4-dichlorophenol; 2.50 $\mu g\ l^{-1}$ for 4-chloro-3-methlyphenol, 4-nitrophenol and pentachlorophenol; and 1.50 $\mu g\ l^{-1}$ for 2,4,6-trichlorophenol and 2,4-dinitrophenol, solutions were made up using ASW to volumes of 0.50, 1, 5 and 18 l.

Derivatization, extraction and solvent evaporation

Phenol acetates were prepared using the following protocol:

Standards of 500 ml and 1 l. Standard phenolic solutions and blanks were prepared with ASW and stored in a 4-l flask, 10 μg of 2,7-dihydroxynaphthalene were added per liter as an internal standard and 8 g of $NaHCO_3$ buffer were added and allowed to dissolve. Two 1-ml aliquots of acetic anhydride were added per liter with vigorous shaking and allowed to react for 2 min. After the reaction, the water sample was poured through Prep-Sep columns under vacuum. The columns were dried with purified nitrogen gas and eluted with 1 ml of ethyl acetate and two 1-ml aliquots of CH_2Cl_2. The extracts were combined, dried over anhydrous sodium sulfate, placed in a conical centrifuge tube, and the volume gently reduced to 100 μl under a vortical stream of purified nitrogen gas. All standards were run in triplicate

Standards of 5 l and 18 l. Standard phenolic solutions and blanks were prepared with ASW and stored in 19-l stainless-steel vessels. Both 10 μg of 2,7-dihydroxynaphthalene as an internal standard and 8 g of $NaHCO_3$ were added per liter as above. Two 1-ml aliquots of acetic anhydride were added per liter with vigorous shaking and allowed to react for 2 min. After the reaction, the sample vessel was pressurized to approximately 130 kPa to force sample through 60-ml C_{18} extraction columns. In addition, the columns were seated in a vacuum manifold to facilitate sample flow. The columns were then dried with purified nitrogen gas and eluted with 5 ml of ethyl acetate followed by two 5-ml aliquots of CH_2Cl_2. The extracts were combined, dried over anhydrous sodium sulfate, and reduced in volume as above. Standards were run in triplicate.

Collection of natural seawater and wastewater samples

Water samples were collected with a 30-l Niskin bottle (White's Point), 4-l amber solvent bottles (Hyperion and JWPCP effluents), or in the 19-l stainless-steel vessels (all other). See Table III for summary of sampling locations and depths. Internal standard, $NaHCO_3$ and acetic anhydride were added as above. The sample vessel was then pressurized to approximately 130 kPa to force sample water through a 0.3-μm pre-combusted glass fiber filter and 60-ml C_{18} extraction columns. The columns were seated in a vacuum manifold as with standards. The columns were then dried and eluted as above. Extracts were reduced to 500 μl.

Calculation of extraction efficiency for p-cresol

[2,6-^{3}H]*p*-Cresol was obtained from Amersham (Arlington Heights, IL, USA), purified by HPLC and added at 500 ng l^{-1} to each of 6 250-ml flasks containing 75 ml of natural seawater collected of the Scripps Institution of Oceanography pier in La Jolla, CA, USA. Three flasks were reacted with acetic anhydride as above to acetylate *p*-cresol. Samples were filtered through 0.3-μm Millipore 25 mm type PH filters to remove cells and the filtrate poured through C_{18} Prep-Sep columns. Effluent from the columns was collected and 1 ml was transferred to scintillation vials. Columns were eluted with 1 ml of MeOH and the eluent transferred to a scintillation vial. Filters, flowthrough water, and column eluent were counted for ^{3}H dpm. Percent efficiency (E) was calculated as:

$$\%E = 1 - \frac{\mathrm{dpm}_{\mathrm{flowthrough}}}{(\mathrm{dpm}_{\mathrm{total}} - \mathrm{dpm}_{\mathrm{filter}})} \times 100\%$$

RESULTS AND DISCUSSION

Gas chromatography–mass spectroscopy

The separation of standards of analyzed phenolic materials is shown in Fig. 1. Shown is the mass spectrum for catechol diacetate. Table I shows characteristic GC–MS data for phenolic standards. Peaks were identified by mass spectral conformational analysis as acetate esters. The molecular ion for catechol is 110 and Fig. 1 shows the addition of two 42 mass unit peaks, 152 and 194. The acetylation allows for identification of phenols based not only on the native mass spectrum (in this case 110 and lower), but also on the addition of the 42 mass unit peak additions. With standards of 0.50 l and varied concentrations, peak area values for each phenolic standard were plotted as a function of concentration. For constant concentration standards, peak area values were plotted as a function of volume. Fig. 2 shows examples for *o*-cresol, *m*-cresol and *p*-cresol. Nitrophenols such as 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol were not adequately derivatized and extracted by this method. The acidity of the $-NO_2$ group may hinder extraction efficiency. For this reason, the nitrophenols were not included in the analysis. During preliminary de-

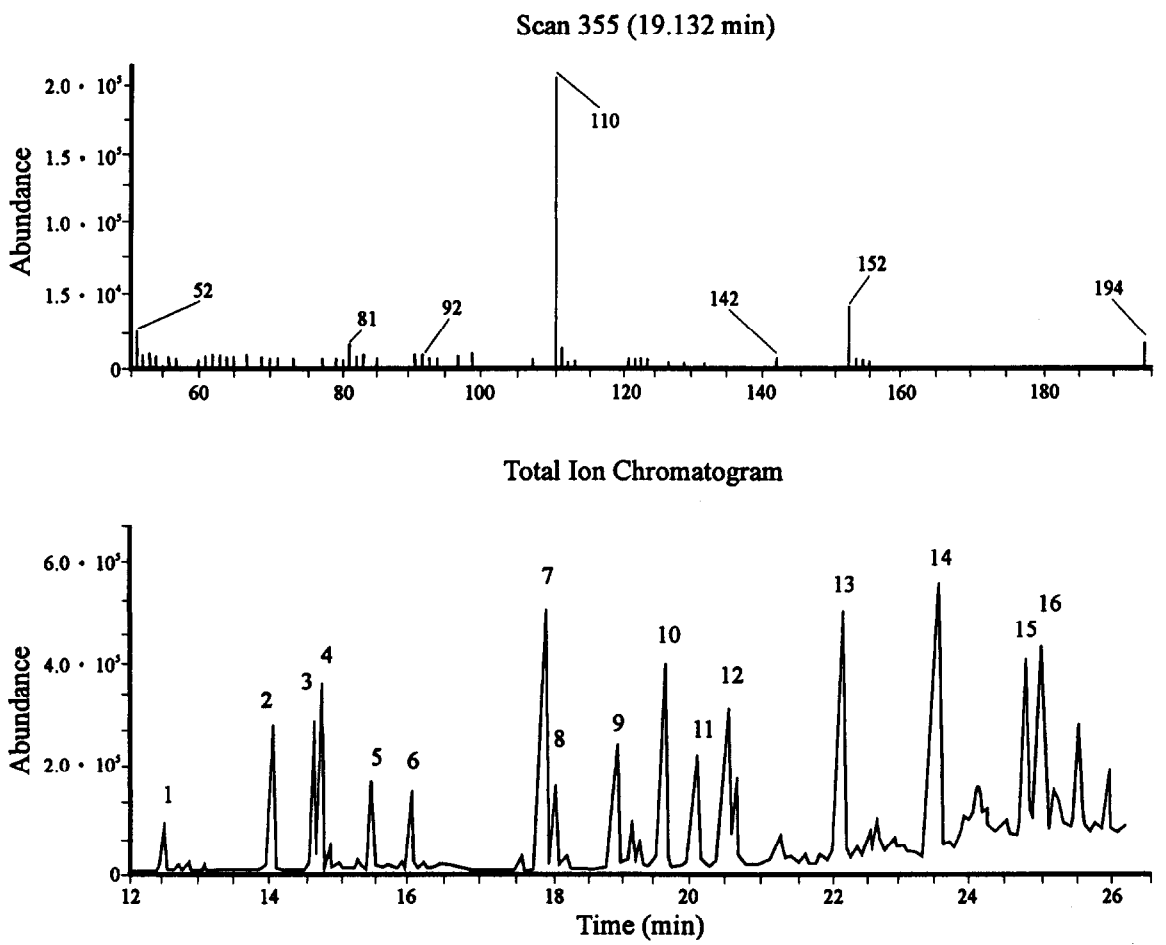

Fig. 1. Total ion chromatogram of standard solution of phenols. Illustrated is scan 355 (19.132 min), mass spectrum for catechol diacetate. Identified phenols (as acetate esters) are: 1 = phenol; 2 = *o*-cresol; 3 = *m*-cresol; 4 = *p*-cresol; 5 = 2-chlorophenol; 6 = 2,4-dimethylphenol; 7 = 4-chloro-3-methylphenol; 8 = 2,4-dichlorophenol; 9 = catechol; 10 = 2,4,6-trichlorophenol; 11 = 3-methylcatechol; 12 = 4-methylcatechol; 13 = internal standard; 14 = diethylphthalate (contaminant); 15 = pentachlorophenol; 16 = phloroglucinol.

TABLE I

CHARACTERISTIC IONS AND RELATIVE ABUNDANCES FOR ACETYLATED PHENOLS

Substrate	Ion (abundance)
Phenol	51 (4), 65 (13), 66 (16), 94 (100), 95 (6), 136 (12)
o-Cresol	51 (10), 77 (22), 79 (13), 107 (40), 108 (100), 150 (14)
m-Cresol	51 (6), 77 (18), 79 (13), 107 (45), 108 (100), 150 (13)
p-Cresol	51 (9), 77 (18), 79 (10), 107 (54), 108 (100), 150 (11)
2-Chlorophenol	63 (14), 64 (11), 73 (11), 128 (100), 130 (33), 170 (11)
2,4-Dimethylphenol	77 (17), 91 (15), 107 (57), 121 (22), 122 (100), 164 (10)
4-Chloro-3-methylphenol	51 (14), 77 (24), 107 (52), 142 (100), 144 (29), 184 (10)
2,4-Dichlorophenol	63 (22), 133 (10), 162 (100), 164 (62), 166 (10), 204 (8)
Catechol	52 (8), 81 (4), 110 (100), 111 (6), 152 (17), 194 (3)
2,4,6-Trichlorophenol	97 (26), 167 (11), 196 (100), 198 (98), 238 (10), 240 (10)
3-Methylcatechol	78 (6), 123 (7), 124 (100), 125 (7), 166 (14), 208 (3)
4-Methylcatechol	78 (6), 123 (8), 124 (100), 125 (7), 166 (13), 208 (3)
Pentachlorophenol	164 (20), 264 (64), 266 (100), 268 (62), 270 (20), 308 (13)
Phloroglucinol	97 (5), 126 (100), 127 (6), 168 (28), 210 (13), 252 (4)

velopment of this method, standard solutions were reduced to pH 2 with HCl prior to extraction in an effort to protonate acidic groups and bolster extraction efficiency. Nitrophenols are able to be resolved, although not quantitatively, over the concentration range used for method development. Severe bubble formation and possible foaming over and loss of the buffered seawater sample may occur with acid addition. Therefore, this manipulation was not incorporated into the final method.

Statistical parameters for the analysis of standards are shown in Table II. The retention time index was calculated by dividing the retention time of the standard phenolic acetate by the retention time of the external standard, butylated hydroxytoluene. A molecular ion peak percent for the parent phenol was calculated by dividing the abundance of the parent molecular ion by the total peak area for all of the standards. The values were averaged and the mean multiplied by 100%. This value was calculated for use with natural seawater extracts which, in some cases, contained compounds that co-eluted within the same retention time period as the analytes. Linear regression analyses were conducted for peak areas plotted against both varying concentration and varying volume (Fig. 2).

Based on analyses of the GC–MS data, the following general formula was used to calculate concentration (x) of phenolics in samples of unknown phenolic composition and concentration:

$$zv^{-1}ynm^{-1}l_{\text{orig}}{}^{-1}V_{\text{fin}} = x \pm s\%$$

where z = correction value for external and internal standards, v = correction value for the use of large volume samples calculated from the deviation between theoretical and actual slope values for peak area *vs.* volume extracted plots (see Fig. 2), y = parent phenol molecular ion peak area, n = parent phenol molecular ion peak percent, m = slope normalized to 1 l of sample (see Fig. 2), l_{orig} = original sample volume, V_{fin} = final extract volume (in μl) and s = percent error calculated by the addition of regression coefficients $(1-r)_{\text{concentration}}$ and $(1-r)_{\text{volume}}$. See linear regression equations in Fig. 2. Table II lists the standard parameters outlined above for standard compounds analyzed in this study.

Analysis of phenolics in natural samples

Water samples were taken from several coastal locations off California (see Table III). In addition, composite effluent from Hyperion and the Joint Water Pollution Control Plant were analyzed. Results are summarized in Table III. Putative identifications are based primarily on mass spectral evidence. Unknown peak spectra

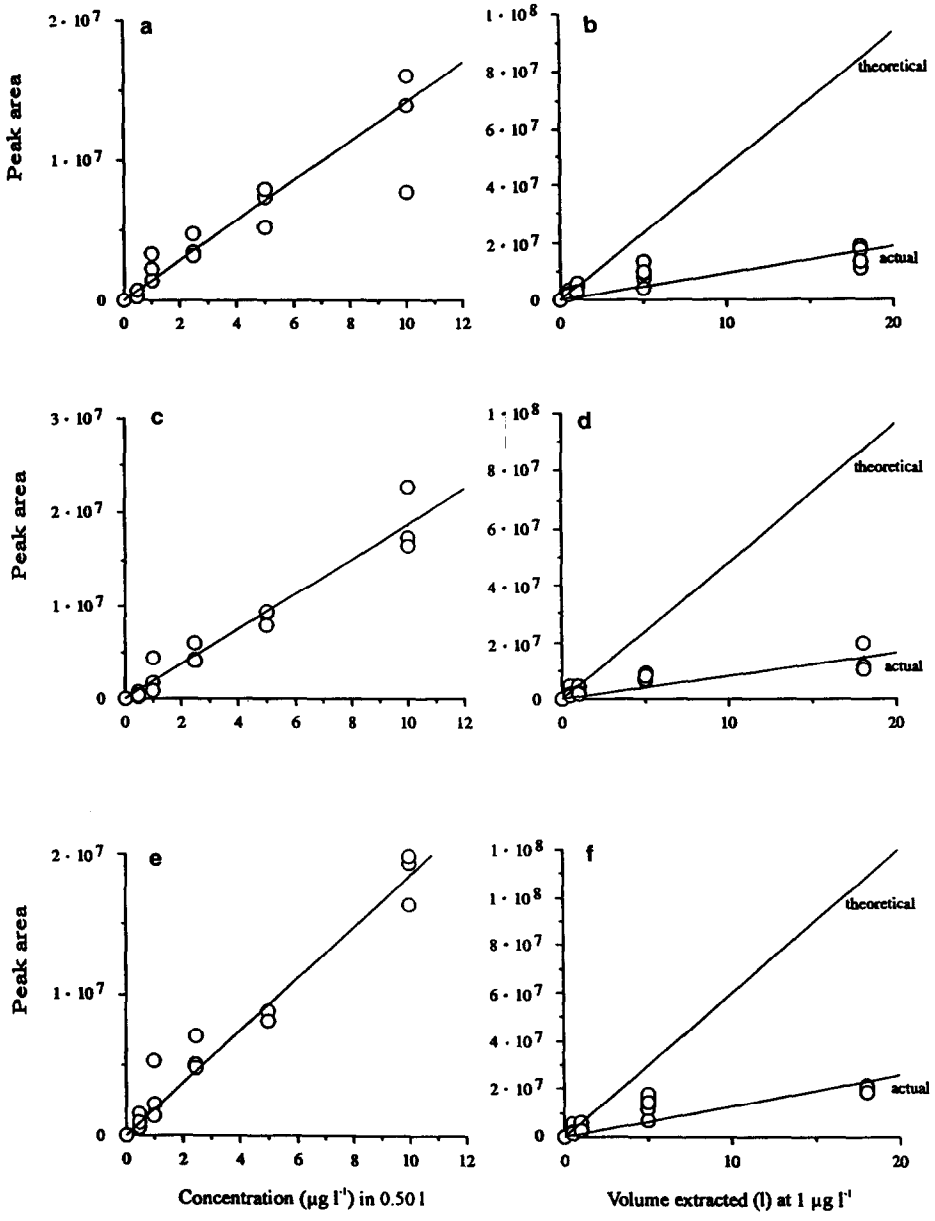

Fig. 2. Peak areas for phenolic standards (a, b) *o*-cresol, (c, d) *m*-cresol and (e, f) *p*-cresol calculated from (a, c, e) constant volume and varied substrate concentration and (b, d, f) constant concentration and varied volume. Theoretical peak area *vs.* volume slope calculated using peak area values for 0.50 l standard volume. Equations: (a) $y = 1.42 \cdot 10^6 x$, $r^2 = 0.98$; (b) $y = 9.33 \cdot 10^5 x$, $r^2 = 0.95$; (c) $y = 1.87 \cdot 10^6 x$, $r^2 = 0.98$; (d) $y = 8.25 \cdot 10^5 x$, $r^2 = 0.94$; (e) $y = 1.86 \cdot 10^6 x$, $r^2 = 0.98$; (f) $y = 1.30 \cdot 10^6 x$, $r^2 = 0.98$.

were searched on the HP 5988 Chemstation spectral data library for suspected matches. Matches were assessed by comparison of molecular ion abundance of the parent phenol and the presence of an $M^+ + 42$ mass unit (acetyl group) peak. Fig. 3 shows a putative identification of 2-methoxyphenol. A library search produced an 80% similarity index with 2-methoxyphenol ($M_r = 124$). The presence of a identifiable peak at 124 + 42 (166) presents strong evidence for

TABLE II

ANALYTICAL AND STATISTICAL PARAMETERS FOR PHENOLIC STANDARDS

Values are used in the generalized equation: $zv^{-1}ynm^{-1}l_{orig}{}^{-1}V_{fin} = x \pm s\%$, where z = correction value for external and internal standards, v = correction value for the use of large volume samples, y = parent phenol molecular ion peak area, n = parent phenol molecular ion peak percent, m = slope normalized to 1 l of sample (see Fig. 4), l_{orig} = original sample volume, V_{fin} = final extract volume (in μl) and s = percent error.

Substrate	Retention time index	Parent phenol molecular ion	Parent phenol molecular ion peak percent (n)	Volume correction (v)	Slope ($m \times 10^6$)	Percent error (s)
Phenol	0.601	94	1.83	1.55	0.62	6
o-Cresol	0.697	108	1.28	0.20	2.84	4
m-Cresol	0.707	108	1.37	0.17	3.74	5
p-Cresol	0.713	108	1.33	0.22	3.72	3
2-Chlorophenol	0.750	128	1.28	0.50	3.10	4
2,4-Dimethylphenol	0.778	122	0.77	1.66	2.92	4
4-Chloro-3-methylphenol	0.867	142	0.71	0.54	4.16	4
2,4-Dichlorophenol	0.880	162	0.94	0.46	3.88	4
Catechol	0.923	110	1.11	0.27	4.90	7
2,4,6-Trichlorophenol	0.957	196	0.51	0.70	3.74	5
3-Methylcatechol	0.981	124	1.14	0.26	3.48	4
4-Methylcatechol	1.010	124	1.15	0.58	5.46	4
Pentachlorophenol	1.230	266	0.42	0.45	2.18	4
Phloroglucinol	1.240	126	0.96	0.41	2.50	9

identification as 2-methoxyphenol. Other phenols identified qualitatively (Table III) were assessed in a similar manner.

Samples were also taken from "pristine" coastal (5 m depth off Scripps Institution of Oceanography pier) and open ocean (Station M, 34°48.51 N × 123°00.86 W) at 200 m sites. No recognizable phenols were detected in these samples (concentrations greater than 100 pg l^{-1}). Brown algae such as *Fucus* spp. are known to contain phenolic materials, generally thought to be polymers of phloroglucinol [21,47,48]. In nearshore environments, degradation of these materials is most likely rapid, perhaps facilitated by solar radiation [40–42] or through pathways that do not include phloroglucinol as an intermediate. Phenolic compounds appear, from the results of this study, to be confined predominantly to nearshore marine environments where terrestrial input is considerable or dilution is hampered by physical enclosure, such as in San Diego Bay.

Concentration ranges for putative identifications were determined by the largest range of deviation from the known standards analyzed, the lowest value from the equation for *o*-cresol and the highest from the equation for 2,4-dimethylphenol. These ranges are only estimates and definitive results require the use of identified compounds as a series of analyzed standards. Phenols identified in this way were all from the Simpson and Louisiana–Pacific outfall sites, and are generally consistent with phenolic compounds previously isolated from pulp mill facilities [9,49,50].

The use of single ion monitoring (SIM) acquisition during GC–MS might improve sensitivity in quantitative analysis, especially if only a narrow range of phenolic materials is suspected to be in a particular sample. For the purposes of the qualitative analysis performed in this work, scan mode was chosen for the widest possible detection ability.

Calculation for derivatization and extraction efficiency

Percent efficiency was calculated using radiolabeled *p*-cresol. This allowed for a direct com-

TABLE III

SAMPLING SITES AND PHENOLS INDENTIFIED

Phenols in italics are putative identifications based on mass spectral data. See text.

Sampling location; coordinates; date	Depth	Phenols identified	Concentration ($ng\,l^{-1}$)
Spanish Landing, San Diego Bay; 32°43′40″ × 117°12′42″; April 4, 1992	Surface	*o*-Cresol	3 ± 0.5
		Catechol	130 ± 21
		3-Methylcatechol	33 ± 5
		4-Methylcatechol	22 ± 3
Sweetwater Channel, San Diego Bay; 32°38′43″ × 117°07′25″; December 9, 1992	11 m	Phenol	228 ± 36
		o-Cresol	5 ± 1
		Catechol	188 ± 30
Simpson Outfall; 40°48′03″ × 124°12′50″; June 24, 1992	Surface	Phenol	32 ± 5
		o-Cresol	22 ± 3
		p-Cresol	15 ± 2
		2-Methoxyphenol	*390–6600*
		2,3,5,6-Tetramethylphenol	*12–200*
		2-Methoxy-4-methylphenol	*17–280*
		4-Ethyl-2-methoxyphenol	*110–1900*
		2-Methoxy-4-isopopenylphenol	*13–220*
		4-Hydroxy-3-methoxybenzaldehyde	*82–1400*
		4,5-Dichloro-2-methoxyphenol	*19–320*
		1,1-Dimethylethylcatechol	*66–1100*
Louisiana-Pacific Outfall; 40°49′05″ × 124°12′10″; June 24, 1992	Surface	Phenol	12 ± 2
		o-Cresol	9 ± 1
		Catechol	100 ± 16
		2-Methoxyphenol	*34–580*
White's Point Outfall 33°41′40″ × 118°19′30″; March 10, 1993	34 m	Phenol	328 ± 52
		o-Cresol	8 ± 1
		p-Cresol	60 ± 10
JWPCP effluent; N/A; March 9, 1993	N/A[a]	Phenol	7000 ± 1120
		o-Cresol	2000 ± 320
		p-Cresol	2400 ± 380
Hyperion effluent; N/A; February 3, 1993	N/A	*o*-Cresol	170 ± 27
		p-Cresol	516 ± 83

[a] N/A = Not applicable.

parison of derivatization–extraction efficiency between unreacted and acetic anhydride derivatized *p*-cresol. Extraction efficiency for derivatized *p*-cresol was calculated at 97% while underivatized *p*-cresol was extracted at 86%. These results are consistent with published efficiencies calculated for the acetate esters of *o*- and *m*-cresol extracted with liquid solvent (CH_2Cl_2) [37]. Derivatizing was necessary in this experimental protocol for the extraction of catechol, 3-methylcatechol, 4-methylcatechol and phloroglucinol. Recoveries were essentially 0% for these unreacted compounds. During optimization of buffer addition, it was noted that under

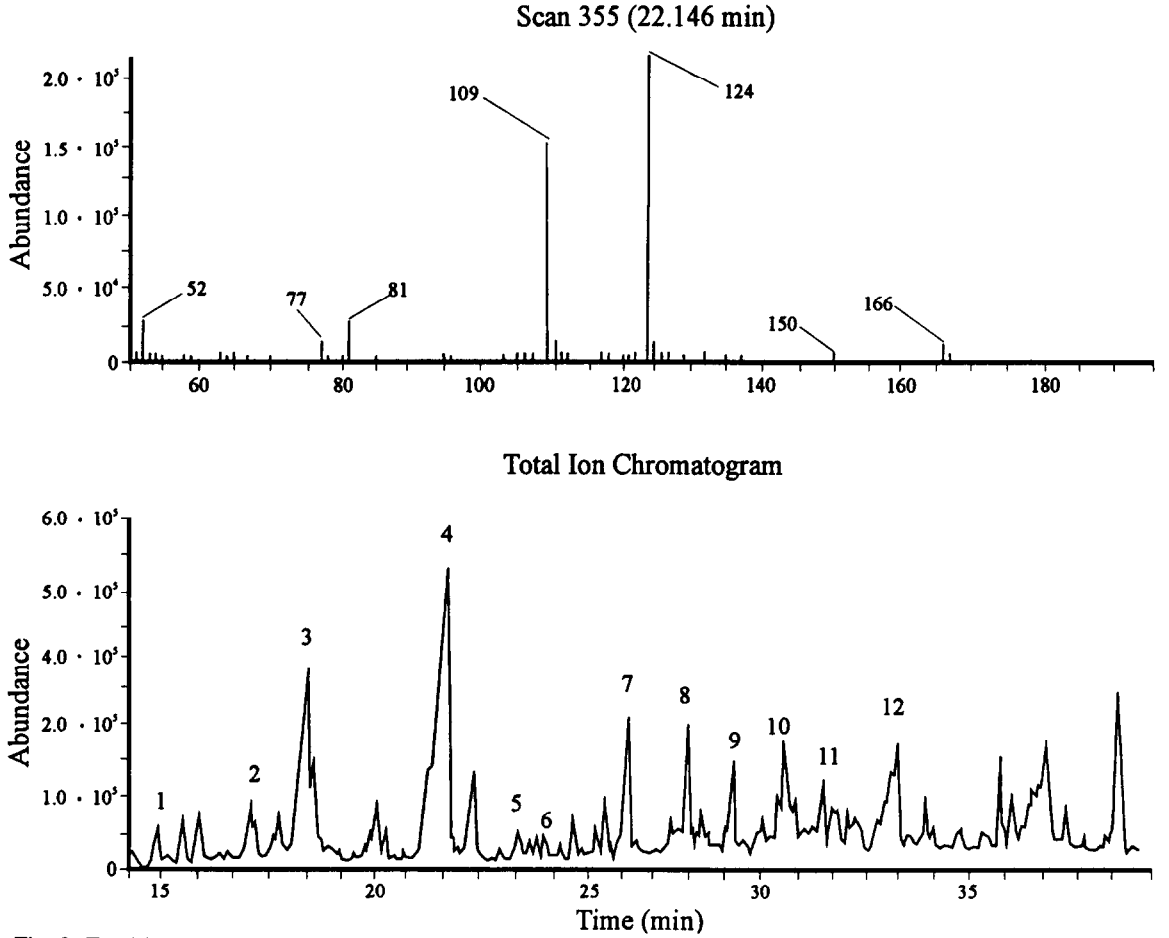

Fig. 3. Total ion chromatogram for Simpson outfall extract. Illustrated is scan 469 (22.146 min), identified as 2-methoxyphenol acetate. Other identified phenols (as acetate esters) are: 1 = phenol; 2 = *o*-cresol; 3 = *p*-cresol; 4 = 2-methoxyphenol (above); 5 = 2,3,5,6-tetramethylphenol; 6 = 2-methoxy-4-methylphenol; 7 = 4-ethyl-2-methoxyphenol; 8 = 2-methoxy-4-isopropenylphenol; 9 = 4-hydroxy-3-methoxyphenol; 10 = 4,5-dichloro-2-methoxyphenol; 11 = 1,1-dimethylethylcatechol; 12 = internal standard.

less than optimal conditions (too little buffer added), acetylation of one hydroxyl group in dihydroxybenzenes occurred allowing for only partial recovery.

CONCLUSIONS

A method is described which uses acetic anhydride for the direct acetylation of phenols in aqueous solutions. The use of this rapid and relatively inexpensive method was optimized for large volume samples using SPE and GC–MS qualitative and quantitative analysis. The advantages of this modification are (1) the ability to extract, qualify and quantify large volumes (tens of liters) of sample suspected of containing phenolic materials; (2) the increased extraction efficiency obtained with aqueous acetylation prior to extraction such that polar phenols such as catechols and phloroglucinol are extractable; (3) the reduction in use of expensive and toxic solvents; and (4) the ability to screen mass spectral data for conformational analysis and putative identification of unknown phenols in a water sample.

ACKNOWLEDGEMENTS

Research was funded in part by a grant from the National Sea Grant College Program, Na-

tional Oceanic and Atmospheric Administration (NOAA), US Department of Commerce, under grant number NA89AA-D-SG138, project number R/CZ-98 through the California Sea Grant College, and in part by the California State Resources Agency. The views expressed herein are those of the author and do not necessarily reflect the views of NOAA or any of its subagencies. The US Government is authorized to reproduce and distribute for governmental purposes. Funds and logistical support were also provided by the Office of Naval Research under grant N00014-87-K-0148 and by the Marine Life Research Group at Scripps Institution of Oceanography. Special thanks are given to the County Sanitation District of Los Angeles County and the crew of the vessel *RV Ocean Sentinel*, the Los Angeles Sanitation District and the crew of the vessel *RV La Mer*, and John D. Faulkner and Russel Vernon-Clark for review and comments on this manuscript.

REFERENCES

1 E.E. Hargesheimer and R.T. Coutts, *Environ. Sci. Technol.*, 5 (1984) 433.
2 D.J. McDermott, *Southern California Coastal Water Research Project Annual Report 1974*, Southern California Coastal Water Research Project, Long Beach, CA, 1974, p. 89.
3 J.N. Cross and D.M. Wiley (Editors), *Southern California Coastal Water Research Project Annual Report 1989–90*, Southern California Coastal Water Research Project, Long Beach, CA, 1990, p. 9.
4 T.W. Federle, *Can. J. Microbiol.*, 34 (1988) 1037.
5 H.A. Schafer, *Southern California Coastal Water Research Project Annual Report 1978*, Southern California Coastal Water Research Project, Long Beach, CA, 1978, p. 97.
6 H.R. Harvey, J.H. Tuttle, R. Dawson and J.T. Bell, in D.A.C. Manning (Editor), *Organic Geochemistry Advances and Applications in Energy and the Natural Environment*, St. Martin's Press, New York, 1991, p. 237.
7 I. Tolosa, J.M. Bayona and J. Albaigés, *Mar. Poll. Bull.*, 22 (1991) 603.
8 J. Perez, T. de la Rubia, J. Moreno and J. Martinez, *Environ. Toxicol. Chem.*, 11 (1992) 489.
9 A. Morales, D.A. Birkholz and S.E. Hrudey, *Water Environ. Res.*, 64 (1992) 669.
10 P. Larsson and K. Lemkemeier, *Water Res.*, 23 (1989) 1081.
11 L. Tranvik, P. Larsson, L. Okla and O. Regnell, *Environ. Toxicol. Chem.*, 10 (1991) 195.
12 J.K. Whelan, M.E. Tarafa and E.B. Sherr, *Org. Mar. Geochem.*, 1986 (1993) 62.
13 G.M. King, *Appl. Environ. Microbiol.*, 54 (1988) 3079.
14 G.M. King, *Nature*, 323 (1986) 257.
15 B. Charriere, F. Gadel and L. Serve, *Hydrobiologia*, 222 (1991) 89.
16 S.M. Steinberg, M.I. Venkatesan and I.R. Kaplan, *Mar. Chem.*, 21 (1987) 249.
17 K. Abrahamsson and S. Klick, *Mar. Pollut. Bull.*, 22 (1991) 227.
18 D.J. Carlson, *Nature*, 296 (1982) 426.
19 D.J. Carlson and L.M. Mayer, *Nature*, 286 (1980) 482.
20 V.N. Kozitskaya, *Hydrobiol. J.*, 3 (1984) 54.
21 P.D. Steinberg, *Oecologia*, 78 (1989) 373.
22 J.McN. Sieburth and A. Jensen, *J. Exp. Mar. Biol. Ecol.*, 3 (1969) 275.
23 R.L. Malcolm, *Anal. Chim. Acta.*, 232 (1990) 19.
24 K.J. Meyers-Schulte and J.I. Hedges, *Nature*, 321 (1986) 61.
25 *Method of Organic Chemical Analysis of Municipal and Industrial Wastewater, US EPA-600/4-83-057*, EPA, Cincinnati, OH, 1982.
26 *Fed. Reg.*, 49, No. 209 (Oct. 26, 1984).
27 E. Tesarová and V. Pacáková, *Chromatographia*, 17 (1983) 269.
28 R.C. Quackenbush, D. Bunn and W. Lingren, *Aquat. Bot.*, 24 (1986) 83.
29 K. Abrahamsson and T.M. Xie, *J. Chromatogr.*, 279 (1983) 199.
30 A. Alfieri, G. Crawford and I. Ahmad, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 760.
31 H.O. Friestad, D.E. Ott and F.A. Gunther, *Anal. Chem.*, 41 (1969) 1750.
32 C. Baiocchi, E. Campi, M. Gennaro, E. Mentasti and P. Mirti, *Chromatographia*, 15 (1982) 660.
33 W.M. Walter, Jr. and A.E. Purcell, *J. Agric. Food Chem.*, 27 (1979) 942.
34 G. Bengtsson, *J. Chromatogr. Sci.*, 23 (1985) 397.
35 L. Renberg, *Chemosphere*, 10 (1981) 767.
36 L. Renberg and K. Lindstrom, *J. Chromatogr.*, 214 (1981) 327.
37 R.T. Coutts, E.E. Hargesheimer and F.M. Pasutto, *J. Chromatogr.*, 179 (1979) 291.
38 H.-B. Lee, L.-D. Weng and A.S.Y. Chau, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 789.
39 T.J. Hendricks, *Southern California Coastal Water Research Project Annual Report 1977*, Southern California Coastal Water Research Project, Long Beach, CA, 1977, p. 41.
40 K. Lin and D.J. Carlson, *Mar. Chem.*, 33 (1991) 9.
41 H. Kawaguchi, *J. Contam. Hydrol.*, 9 (1992) 105.
42 H.M. Hwang, R.E. Hodson, R.F. Lee, R.G. Zika and W.J. Cooper, *Photochem. Environ. Aquat. Syst.*, 327 (1987) 27.
43 A.V. Palumbo, F.K. Pfaender and H.W. Paerl, *Environ. Toxicol. Chem.*, 7 (1988) 573.
44 T.J. Boyd and A.F. Carlucci, *Aquat. Toxicol.*, 25 (1993) 71.
45 G.W. Bartholomew and F.K. Pfaender, *Appl. Environ. Microbiol.*, 45 (1983) 103.

46 National Ocean Pollution Program Office, *Federal Plan for Ocean Pollution Research, Development, and Monitoring; Fiscal Years 1992–1996*, US Department of Commerce, Washington, DC, 1991, p. 9.
47 D.J. Carlson and L.M. Mayer, *Can. J. Fish. Aquat. Sci.*, 40 (1983) 1258.
48 J. Tuomi, H. Ilvessalo, P. Niemela, S. Siren and V. Jormalainen, *Botanica Marina*, 32 (1989) 505.
49 M.M. Haeggblom, J.H.A. Apajalahti and M.S. Salkinoja-Salonen, *Wat. Sci. Tech.*, 20 (1988) 205.
50 J.L. Sarmiento and E.T. Sundquist, *Nature*, 356 (1992) 589.