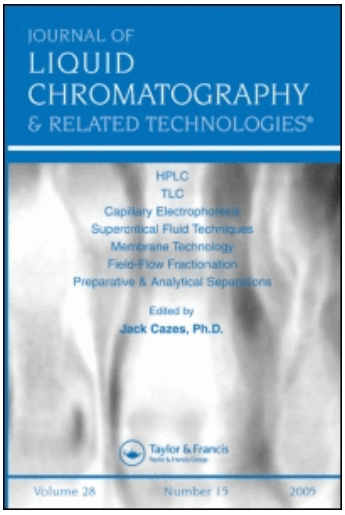

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Determination of Benzoic Acid, Chlorobenzoic Acids and Chlorendic Acid in Water

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DETERMINATION OF BENZOIC ACID, CHLOROBENZOIC ACIDS AND CHLORENDIC ACID IN WATER

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ABSTRACT

The title compounds were isolated from acidified (pH 1) water by extraction with methyl t-butyl ether. Analytes were concentrated by back-extracting the ether with 0.1 N sodium hydroxide which was separated and acidified. This solution was analyzed by C_{18} reversed-phase HPLC with water/acetonitrile/acetic acid eluent and UV detection at 222 nm. The method has detection limits of 200 μg/L for chlorendic acid and 100 μg/L for benzoic acid and each isomer of chlorobenzoic acid. Validation studies with water which was fortified with the analytes at concentrations ranging from one to ten times detection limits resulted in average recoveries of >95%.

INTRODUCTION

To characterize and conduct treatment studies of a landfill leachate an analysis procedure was required to determine concentrations of benzoic acid, the three isomers of chlorobenzoic acid and chlorendic acid (1-3). Without derivatization, acids are not good candidates for GC analysis. Even with derivatization, the chlorobenzoic acid isomers are poorly resolved (4,5); therefore, an HPLC procedure was sought.

Methods for determining urinary acids have been reported using reversed-phase HPLC with acetonitrile/water and phosphoric acid as an eluent modifier (6,7). Using similar HPLC conditions, separation of benzoic acid and o-chldorobenzoic acid has been reported; however, m- and p-chlorobenzoic acids coeluted (8). For chlorendic acid, no literature information was found to provide guidance in developing an HPLC method. Organic acids have been analyzed by reversed-phase HPLC using acetic acid as an elutent modifier (7,9). Our investigations of this modifier with a water/acetonitrile gradient provided resolution of all target compounds on a 4.6 mm i.d. C_{18} column when operated at 2 mL/min. Although component resolution was sufficient for reliable quantitative results, the quality of chromatographic traces was degraded by peak tailing for m- and p-chlorobenzoic acids and chlorendic acid.

For sample preparation, a liquid-liquid extraction was developed. Ethyl ether and ethyl acetate, either alone or in combination, have been used to extract organic acids from acidified urine samples (6,10) and ethyl ether is specified for extracting chlorinated phenoxy acid herbicides from acidified water samples (11). We found that three extractions of acidified (pH 2) sample with methyl t-butyl ether (MTBE) gave good recoveries of target compounds. These extracts were combined, then an aliquot was analyzed without further treatment. Since most samples contained high concentrations of analytes, their 15-fold enrichment in extracts was satisfactory to meet a 2 ppm detection limit requested by our investigators.

For testing groundwater around the landfill and for monitoring discharges of treated waters, regulatory agencies mandated detection limits of 200 μg/L for chlorendic acid and 100 μg/L for other acids. To achieve these detection limits and improve chromatography, method modifications were necessary. This paper describes that development work and presents method validation results.

EXPERIMENTAL

Instrumentation and Conditions

The liquid chromatograph was a Hewlett Packard model 1090M and was equipped with a ternary solvent delivery system, column oven, automatic liquid sampler with variable-volume injector, and a 1040 diode array detector. The system was operated with a 79994 chemstation with revision 5.22 software, used a Hewlett Packard disc drive, and recorded data with a Hewlett Packard 2225 Thinkjet printer. Analysis conditions were:

Column	-	Waters Nova-Pak C_{18} (2 X 150 mm with 4 μm particle diameter)
Sample Vol.	-	20 μl
Temperature	-	Column oven was set for 40 °C
Eluent Flow	-	0.25 mL/min.
Eluent	-	From 0 to 10 min. elution solvent was composed of 11% acetonitrile/64% water/25% water which contained 2 mL/L acetic acid; between 10 and 45 min. composition changed linearly to 50% acetonitrile/25% water/25% water with 2 mL/L acetic acid. This composition was held for five min.
Run Time	-	50 min.
Post Time	-	After run termination, a 15 min. hold time was in effect to allow solvent equilibration to initial composition.
Detector	-	Set for 222 nm with a 10 nm window and a reference wavelength of 550 nm with 80 nm window. A UV spectrum of each

chromatographic peak was automatically recorded from 210 to 400 nm. Detector run time was 45 min.

Data Reduction - Using a peak width of 0.2 min and threshold of 2, the detector signal was integrated and reported in areas units.

Reagents and Supplies

Water -- Purified, Millipore Milli-Q System

Acetonitrile -- Burdick & Jackson, UV grade, Cat. # 015-4

Methanol -- Burdick & Jackson, Cat #230-4

Methyl t-butyl ether -- EMScience, Omni Solv®

Acetic Acid, Glacial -- EMScience, Tracepur® Cat. AX00731-1

Sulfuric Acid -- Baker Analyzed, Ultrex® Ultrapure Reagent Cat. 4802-05

Sodium Hydroxide -- Pellets, GR from EMScience

pH Indicator Strips -- EM Colorphast®, 0-2.5 range, Cat. 9580

Extraction Flasks -- 110 mL Cassia Volumetric Flasks

Centrifuge Tubes -- Corning, Pyrex, 15 mL, Cat. 8084

Teflon Stoppers -- to fit above glassware

Benzoic Acid -- Baker Analyzed Reagent ACS grade, Cat. 0076-01CSA

o-Chlorobenzoic Acid -- Aldrich 98%, Cat. 13,557-7

m-Chlorobenzoic Acid -- Aldrich 99+%, Cat. C2,460-4

p-Chlorobenzoic Acid -- Aldrich 99%, Cat. 13,558-5

Chlorendic Acid -- Pfaltz & Bauer, Cat. C07970

Syringe Filters -- Gelman, Acrodisc® 0.45 μm, 3 CR PTFE

Primary Analyte Solutions

In 100-mL volumetric flasks, aqueous solutions were prepared by partially dissolving 0.100 g of each acid (0.200 g of chlorendic acid) in 10 mL

of 0.1 N NaOH. Aliquots of water were successively added with vigorous mixing until each acid dissolved then the volume was adjusted to 100 mL.

Stock Analyte Solution

A solution containing 100 μg/mL of each acid (200 μg/mL for chlorendic acid) was prepared by combining 10-mL aliquots of each primary solution then diluting this mixture to 100 mL with water. Other concentrations of stock solution were prepared in a similar way by diluting 7.5, 5.0, 2.5 and 1.0-mL aliquots of primary solutions.

Calibration Solutions

Aqueous calibration solutions were prepared by diluting 5.0 mL of each stock solution to 10 mL with 0.02 N H_2SO_4. For methanolic calibration solutions, 5.0 mL of each stock solution was added to a 10-mL volumetric flask along with 2 mL of 0.05 N H_2SO_4 and 3 mL of methanol. Upon mixing there was a small volume reduction which was corrected by adding a few drops of methanol.

Preparation of Fortified Solutions

To a Cassia flask 1.0 mL of a stock solution was added which then was diluted to 100 mL with either 0.1 N H_2SO_4 or water samples which had been adjusted to pH 1.

Sample Extraction

Samples were at room temperature and pH was adjusted to 1 using a 0-2.5 pH strip to check acidity. A 100-mL aliquot then was added to an extraction flask. To samples as well as any fortified solutions 11 mL of MTBE was added and vigorously mixed to extract the water. After phase separation only about 5 mL of MTBE phase resulted due to a 5% solubility

loss of MTBE and solution volume reduction from phase mixing. Loss of MTBE was a good indicator that extraction had been sufficiently vigorous. Therefore, if noticeably more that 5 mL was recovered, extraction was continued. The MTBE layer was withdrawn and placed in a centrifuge tube. The extraction was repeated using 6 mL of MTBE. After phase separation, the resulting 6 mL of MTBE was combined with that from the first extraction. To the recovered MTBE 1.0 mL of 0.1 N NaOH was added and briskly mixed. Extraction could not be overly vigorous otherwise a suspension formed that slowly separated. Appropriate agitation was indicated by formation of small caustic droplets which quickly settled. Following phase separation as much MTBE as possible was withdrawn and discarded. Any remaining MTBE layer and dissolved MTBE was eliminated using a stream of nitrogen. Completion of this step resulted in 1.0 mL of solution as confirmed by graduation marks on the centrifuge tube. Now 1.0 mL of 0.12 N H_2SO_4 was added except for extracts from samples known or suspected tocontain >1 ppm of p-chlorobenzoic acid or >7 ppm m-chlorobenzoic acid. For these, 1.0 mL of a 40/60 mixture of 0.3 N H_2SO_4/ methanol was added. Reasons for using acidic methanol are given later under Results and Discussion. These solutions were then examined by HPLC without prior filtration; however, filtration using an 0.45-μm PTFE membrane filter was acceptable (see results and discussion).

Calculation of Analyte Concentrations and Recoveries

Sample area responses were compared with area responses produced by calibration solutions. Samples responses should be within the calibration range otherwise extracts need diluted or a fresh sample aliquot should be diluted and extracted. Another approach is to inject less sample into the HPLC, e.g., 10, 5, 2, or 1 μL.

Fortified solutions were prepared by diluting 1.0 mL of stock solution to 100 mL. Since sample preparation provides an enrichment factor of 50,

an extract matched a calibration solution made from the same stock solution when 100% recovery was achieved. Therefore:

$$\% \text{ Recovery} = \frac{\text{Area Response of Extract}}{\text{Area Response Cal. Soln.}} \times 100$$

RESULTS AND DISCUSSION

This work aimed to develop an analysis with validated method detection limits of 100 μg/L for each analyte (200 μg/L for chlorendic acid); these limits were about 20 times lower than the method then in use. To achieve this goal, the HPLC analysis and sample extraction procedure were investigated. Using a microbore (2 mm i.d.) HPLC column method sensitivity was increased. However, with a 0.25 mL/min eluent flow, only 2 μL of MTBE could be injected due to its insolubility in the eluent. A 2 mL/min flow previously used on a standard (4.6 mm i.d.) HPLC column allowed MTBE to quickly disperse and dissolve so injecting 20 μL had been acceptable. Therefore, to provide a different extract solvent and to lower the method detection limit, the sample extraction procedure was modified to include a caustic back-extraction of MTBE extracts. In this way analytes were concentrated into an aqueous solution which was made compatible for HPLC by pH adjustment.

Step one in a sample analysis involves MTBE extraction of acidified water. By adjusting the pH to 1, only two extractions are needed to obtain >90% recoveries for each analyte. Effect of pH on extraction efficiency is presented in Table 1. The data are from a single extraction of samples with 1 ppm of each analyte (2 ppm for chlorendic acid) followed by extract workup and analysis.

Step two of sample preparation concentrates and transfers target acids into a sodium hydroxide solution while providing an extract cleanup *mechanism*. Since MTBE can extract other sample components, a caustic

TABLE 1

Solute Recoveries - pH Effect on MTBE Extraction of Water

Solute	% Recovery*						
	pH 1	pH 3	pH 5	pH 7	pH 9	pH 11	pH 13
Benzoic Acid	75	75	49	38	19	-	-
o-Chlorobenzoic Acid	86	59	8	5	2	-	-
m-Chlorobenzoic Acid	92	92	75	68	44	-	-
p-Chlorobenzoic Acid	91	92	76	71	49	-	-
Chlorendic Acid	93	90	73	59	16	-	-

* A dash indicates no response greater than twice the background signal was observed.

TABLE 2

Effect of Extraction Vigor on Recovery

	% Recovery *		
Solute	Extraction A	Extraction B	Extraction C
Benzoic Acid	96	73	84
o-Chlorobenzoic Acid	95	74	81
m-Chlorobenzoic Acid	101	74	87
p-Chlorobenzoic Acid	100	74	87
Chlorendic Acid	95	58	72

* Only one analysis for each extraction technique:

A - The procedure as detailed in this report.
B - Vigorous MTBE extraction with very gentle caustic extraction.
C - Gentle MTBE extraction with caustic extraction as described in this report.

back-extraction assures that only acidic compounds will be present in the final extracts. Table 1 indicates that a pH>11 will completely retain analytes in the aqueous phase. With 0.1 N NaOH (pH 13), total recovery is expected. Even if traces of H_2SO_4 are entrained in the ether from the MTBE extraction, it is unlikely that this would cause the caustic pH to fall below 11.

As shown in Table 2, vigor of phase mixing during MTBE and NaOH extractions is important. The data represents results from analyses of water which contained each analyte at 1 ppm (2 ppm for chlorendic acid). Significant recovery losses were evident when either extraction was not sufficiently vigorous. Interestingly, recovered MTBE from the gentle

extraction was about 1 mL more than for the two more vigorous analyses. As noted in the extraction process description, volume of MTBE collected after extraction parallels extraction vigor.

The final preparation step requires neutralization/acidification of the caustic extract and its volume adjustment to 2.0 mL. For this, sufficient H_2SO_4 is added to produce a solution pH of 2. The extract pH is important for providing good chromatographic behavior for o-chlorobenzoic acid. Acidity of eluents markedly influences retention times for acidic compounds (12-16). For this analysis, o-chlorobenzoic acid is especially sensitive to eluent pH. When extract is injected into the HPLC column, eluent pH momentarily changes. Until the pH returns to initial conditions the o-chloroenzoic acid migration depends on the pH upset. When extracts had low acidity (pH 6-7), o-chlorobenzoic acid eluted quickly and exhibited a broadened response; in some cases peak splitting occurred. By reducing extract pH the peak sharpened and eluted with increased retention times which became reproducible around pH 2-3. Therefore, to assure acceptable HPLC performance the extract pH was adjusted to 2. An example of column performance is provided by Figure 1 which is for a calibration solution representing five times the method detection limit.

Each analyte exhibited linear response to the UV detector from half to 20 times the method detection limit; correlation coefficients were > 0.999. Samples responding above the calibrated range should be diluted or a smaller amount of extract can be injected if the injector device is accurate. We tested injector accuracy by injecting 20, 10, 5, 2 and 1 μl of a calibration solution containing each analyte at 50 μg/mL (100 μg/mL for chlorendic acid). Linearity of response for each compound was observed with correlation coefficients of > 0.999.

A diode array detector is advantageous in that a complete UV spectrum can be automatically recorded for each observed peak. Comparison of each spectrum with a library reference is useful for confirming peak identities (17,18); a practice we routinely apply for field sample analyses.

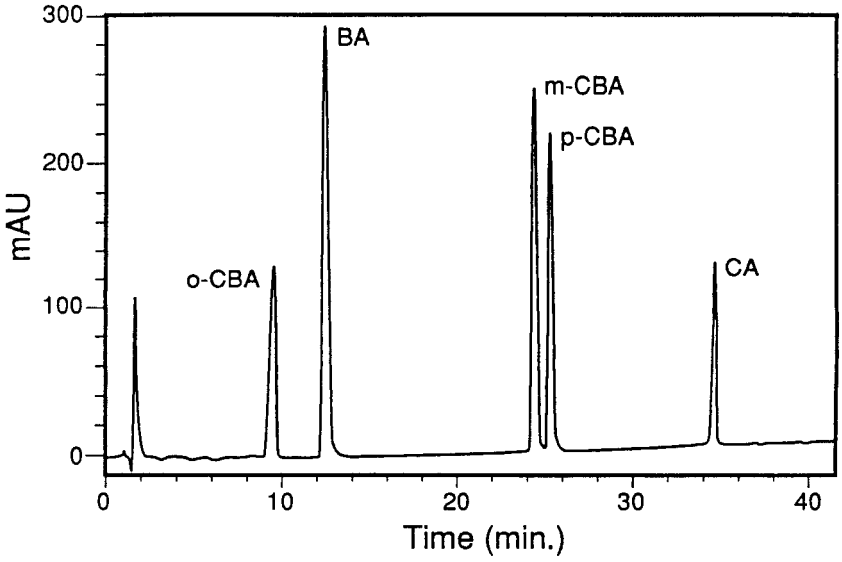

FIGURE 1. Chromatogram for a calibration solution of benzoic acid (BA), o-chlorobenzoic acid (o-CBA), m-chlorobenzoic acid (m-CBA), p-chlorobenzoic acid (p-CBA), and chlorendic acid (CA). Chlorenid acid is 50 μg/mL and the other acids are 25 μg/mL.

Extract filtration prior to analysis may be needed occasionally; however, it must not alter extract composition. As presented in Table 3 a variety of filter materials was tested. All except a PTFE membrane caused analyte loss. The PTFE filter exhibited quantitative transfer of all analytes even for a solution representing the method detection limit.

Water solubility data for chlorendic acid (19) and the other acids (20) reveal that m- and p-chlorobenzoic acid are the least soluble. In acidified aqueous extracts we found the solubilities of m- and p-chlorobenzoic acid decrease to only 350 and 50 ppm respectively. This limits the analytical procedure to samples containing <7 ppm m-chlorobenzoic acid and around 1 ppm for p-chlorobenzoic acid, otherwise addition of aqueous H_2SO_4 to caustic extracts will precipitate those analytes. This would require sample

TABLE 3

Analyte Recovery After Filtration

Normalized Area Response (a,b)									
Compound	No Filter	I.C.	N.S.	PVDF	PF	PTFE	Nylon	PTFE (c)	PTFE (d)
Benzoic Acid	1.00	0.89	0.99	0.95	0.95	0.99	0.86	1.00	1.01
o-Chlorobenzoic Acid	1.00	0.94	1.00	0.94	0.96	0.99	0.81	0.99	1.00
m-Chlorobenzoic Acid	1.00	0.73	0.97	0.87	0.88	1.00	0.57	1.00	1.00
p-Chlorobenzoic Acid	1.00	0.74	0.97	0.89	0.88	0.99	0.60	1.00	1.00
Chlorendic Acid	1.00	0.92	0.86	0.86	0.76	0.99	0.0	0.99	0.99

a) Results using 50 (100) μg/mL methanolic calibration solution.
b) Filter codes mean:
   I.C. - Gelman, Acrodisc, 0.45 μm Ion Chromatography
   N.S. - Gelman, Acrodisc, 0.45 μm Non-sterile
   PVDF - Gelman, Acrodisc PVDF, 0.45 μm
   PF - Gleman, Acrodisc PF, 0.8 μm prefilter with 0.2 μm Supor
   PTFE - Gelman, Acrodisc CR PTFE, 0.45 μm
   Nylon - Baker, SPE, 0.45 μm
c) Results using 5 (10) μg/mL methanolic calibration solution; i.e., detection limit solution.
d) Results using 5 (10) μg/mL aqueous calibration solution.

TABLE 4

Data for Recovery of Benzoic Acid (BA), o-Chlorobenzoic Acid (o-CBA), m-Chlorobenzoic Acid (m-CBA), p-Chlorobenzoic Acid (p-CBA) and Chlorendic Acid (CA) in Spiked Milli-Q Water

	% Recovery (b) - Day 1				
Spike Concentration (a)	BA	o-CBA	m-CBA	p-CBA	CA
1.0 (2.0) µg/mL	94	95	98	99	95
1.0 (2.0) µg/mL	92	93	97	96	97
0.75 (1.5) µg/mL	96	97	99	98	94
0.50 (1.0) µg/mL	93	93	97	97	92
0.25 (0.5) µg/mL	95	93	101	101	90
0.10 (0.2) µg/mL	95	102	97	97	97
	% Recovery (b) - Day 2				
1.0 (2.0) µg/mL	96	95	101	100	94
0.75 (1.5) µg/mL	100	100	105	105	99
0.75 (1.5) µg/mL	92	91	97	97	91
0.50 (1.0) µg/mL	93	93	99	99	93
0.25 (0.5) µg/mL	93	92	98	99	90
0.10 (0.2) µg/mL	92	92	98	98	95
	% Recovery (b) - Day 3				
1.0 (2.0) µg/mL	99	99	103	103	97
0.75 (1.5) µg/mL	96	94	101	100	95
0.50 (1.0) µg/mL	98	97	104	106	94
0.25 (0.5) µg/mL	90	86	95	96	87
0.10 (0.2) µg/mL	95	92	103	102	92
0.10 (0.2) µg/mL	93	92	102	99	91

a) Concentrations in parentheses are for chlorendic acid.
b) Extracts and calibration solutions contained methanol according to optional procedure.

TABLE 5

Data for Recovery of Benzoic Acid (BA), o-Chlorobenzoic Acid (o-CBA), m-Chlorobenzoic Acid (m-CBA), p-Chlorobenzoic Acid (p-CBA) and Chlorendic Acid (CA) in Spiked Field Samples

Field Location A #C92-2470	% Recovery (b)				
Spike Concentration (a,d)	BA	o-CBA	m-CBA	p-CBA	CA
0.25 (0.5) µg/mL	100	100	101	101	104
0.50 (1.0) µg/mL	96	97	100	100	103
Field Location B #C92-2439	% Recovery (c)				
Spike Concentration (a,d)	BA	o-CBA	m-CBA	p-CBA	CA
0.25 (0.5) µg/mL	98	100	99	100	97
0.50 (1.0) µg/mL	93	97	97	97	97

a) Concentrations in parentheses are for chlorendic acid.
b) Extracts and calibration solutions contained methanol according to optional procedure.
c) Extracts and calibration solutions were 100% aqueous.
d) Analysis of unspiked samples resulted in no background concentrations of analytes.

dilution and reanalysis. An optional procedure includes 30% methanol in extract solutions which increases p-chlorobenzoic acid solubility to 300 ppm; all other acids are soluble to > 500 ppm. In this way the method can be extended to samples with 6 ppm of p-chlorobenzoic acid and at least 10 ppm of the other acids.

Methanol in extracts affects the chromatography by reducing analyte retention times except for chlorendic acid. Shifting occurs because the injection solution is a stronger elution solvent than that of the initial HPLC conditions. Peak shape and resolution are not affected. A disadvantage to having analytes in acidified methanol is formation of chlorendic acid monomethyl ester. This was shown when two-day old calibration solutions were reanalyzed after being maintained at room temperature. A new response was observed after the chlorendic acid peak; its area was about 1-2% of the chlorendic acid peak. By day four, it had become 5% and after a week had grown to 8%. A two-week old solution exhibited a peak with 17% the area of chlorendic acid. Due to unwanted esterification of chlorendic acid, the optional procedure should be used only when needed and sample extracts should be chromatographed within a few days after preparation.

Recovery results which were obtained at five concentrations on three separate days using Milli-Q water spikes are given in Table 4. Each day a duplicate sample also was analyzed. Spiked samples containing 1 ppm of p-chlorobenzoic acid had to be analyzed using the optional methanolic procedure. To obtain comparable data for the other spiking levels, calibration soluitons and all extracts were prepared with methanol. Two field samples, each spiked at two concentrations, were included with this study. One set was analyzed using aqueous extracts while the other used methanol addition to extracts. These recovery data are presented in Table 5. The mean recovery of all the Milli-Q water spikes is 96.3% with a relative standard deviation of 4.1% These results and > 98% average recovery average for actual field samples validate the sample extraction and HPLC procedures presented in this paper.

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