Extraction of Phenol and Its Metabolites from Aqueous Solution

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In the development of an extraction method utilizing the "salting out" technique, the extraction efficiencies for phenol, catechol, resorcinol, and hydroquinone from aqueous solutions were determined using tetrahydrofuran (THF), acetonitrile (ACN), dimethylformamide (DMF), ethyl acetate (EtOAc), and diethyl ether (Et_2O). Single additions of potassium fluoride (anhydrous), sodium chloride, lithium chloride, ammonium acetate, and sodium bicarbonate were used to saturate the solvent/water combinations. Phase separations were not observed for combinations of THF/LiCl, $NaHCO_3$; ACN/$NaHCO_3$; and DMF/NaCl, LiCl, NH_4OAc, $NaHCO_3$; ACN/KF converted hydroquinone to quinone, while extraction DMF/KF yielded no hydroquinone in nonacidified solutions. Essentially all other solvent/salt combinations gave quantitative recoveries. As an aside and in addition, KF was determined to be an efficient drying agent.

As part of an ongoing pharmacokinetic investigation of the teratogenicity of phenol and its metabolites (1,2-, 1,3-, and 1,4-dihydroxybenzene) in pregnant Sprague-Dawley rats (Kavlok et al., 1991; Oglesby et al., 1992), there was need of an analytical procedure sufficiently sensitive to provide quantitative data to the parts-per-billion level or better in urine, plasma, and fetal tissue. Utilizing capillary column gas chromatography (GC) and an electron capture detector, the derivatization procedures had to quantitatively yield a product containing fluorine atoms for enhanced electron capture sensitivity and of sufficient stability to endure the long periods of storage required for autosampler injection. The procedure also had to produce minimal reaction with naturally occurring compounds present in the biological media.

After an extensive investigation of the preparation and stability of the products produced by perfluorobenzoyl chloride, pentafluorobutyric anhydride, and perfluorobenzyl bromide, the procedure adopted utilized the reaction of the phenols with bis-3,5-(trifluoromethyl)-benzyl bromide in the presence of potassium carbonate catalyzed by 18-crown-6, similar to the procedure described by Davis (1977). The procedure was modified to accommodate the solvent used to quantitatively extract the phenols from the biological medium (Jones et al., 1993), and it is to this extractive isolation that this paper is addressed.

Several publications have appeared describing extraction procedures for isolating phenol and its dihydric metabolites from aqueous biological media. The benzene metabolites produced by mice (phenol, catechol, and hydroquinone) were extracted from acidified (pH 3) mouse urine with diethyl ether in recoveries of 90%, 100%, and 98%, respectively, as previously reported by Gad-El Garim et al. (1985). Fell and Lee reported a 90% recovery of phenol, catechol, resorcinol, and hydroquinone from sodium chloride saturated neutral or acidic urine solutions by extracting three times with diethyl ether followed by three times with ethyl acetate (Fell and Lee, 1976). Benzene has been described as extracting phenol from water with a 76–109% efficiency, but resorcinol and catechol were obtained in only 2% efficiency (Lamporski and Nestrick, 1978). Using a variety of solvents to extract monohydric alkylphenols from acidified water, it has been determined that diethyl ether gave the highest recoveries for the alkylated phenols (70–75%) but only 48% for phenol itself (Hrivnak and Stelac, 1984). Dihydric phenols were not studied. In analyzing phenol and a series of substituted phenols in aqueous solution, the efficiency of extraction from an acidified (pH 3) or sodium bicarbonate buffered (pH 8.5) solution was determined using a variety of organic solvents; toluene was found to be the most efficient extraction solvent, the percent recovery of phenol being 66% from acidified solutions and 83% from the buffered solution (Bengtsson, 1985).

A recent publication on the "salting-out" of miscible solvents for extraction of neutral polar organic compounds (Legget et al., 1990) prompted an in-depth investigation of this technique for the extraction of phenol and its dihydric metabolites from aqueous biological media. This reported procedure involved the addition of 130 g of sodium chloride to a solution of 100 mL of acetonitrile in 400 mL of solute-containing water, producing a final volume of 518 mL aqueous and 22.0 mL acetonitrile. The compounds used as probes were organic nitrates, nitro compounds, and azines with near quantitative recoveries as quantified by liquid chromatography.

In the present study, a variety of solvents and inorganic salts were investigated for the extraction of 1-mL aqueous samples (neutral and acidified) containing 5 mg/mL of each phenol, and the efficiency was determined by GC analysis using 2,6-dimethylphenol as an internal standard. The results are reported herein.

EXPERIMENTAL PROCEDURES

Dimethylformamide (DMF) was certified ACS (Fisher Scientific) and redistilled the day of use. Acetonitrile (ACN) (Fisher Scientific), ethyl acetate (EtOAc) (Baker), and tetrahydrofuran (THF) (Fisher Scientific) were of residue analysis grade. THF and diethyl ether (Et_2O) (reagent ACS, Fisher Scientific) were fractionally distilled from sodium/benzophenone (Riddick and Bunger, 1970) and used immediately.

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Salts and suppliers used herein were sodium chloride, certified ACS (Fisher Scientific); lithium chloride GR (EM Science); ammonium acetate, certified ACS (Fisher Scientific); potassium fluoride anhydride, 99% (Aldrich); and sodium bicarbonate, certified ACS (Fisher Scientific). The potassium fluoride was stored in a desiccator or an oven (110 °C).

Phenol, catechol, resorcinol, hydroquinone, and the internal standard (IS) 2,6-dimethylphenol were obtained from Aldrich, analyzed by GC for purity, and recrystallized from suitable solvents if necessary. The compounds were stored in brown bottles in a refrigerator at 4 °C.

Reference Standards. Using THF as the example solvent (other extracting solvents were used similarly), triplicate master solutions containing 100 mg/mL of each phenol were prepared by dissolving combined, accurately weighed 500-mg amounts of each phenol and making to 5 mL with redistilled THF. To prepare standards for use in detector response (DR), response factor (RF), and extraction efficiency determinations, 0.5 mL of the master solution was diluted to 10 mL with THF. One milliliter of this solution and 1 mL of the internal standard (IS) solution were then diluted to 10 mL with THF, giving a final concentration of 0.5 mg/mL for each phenol. The same IS solution, prepared by making 100 mg of 2,6-dimethylphenol to 25 mL with appropriate solvent, was used for all standard solutions and extracts for a given salt/solvent combination.

Standards using acetonitrile and distilled dimethylformamide were diluted in the same manner except that the master solutions contained 50 mg/mL of each phenol, yielding 0.25 mg/mL reference standards. For the water-immiscible solvents of diethyl ether and ethyl acetate, 50 mg/mL THF master solutions were prepared and diluted to 0.25 mg/mL with respective solvent as described.

For each solvent, 0.5 mL of the same master solution used in the preparation of the reference standard was diluted to 10 mL with HPLC grade water, yielding aqueous standards for use in extraction studies. Master solutions, reference standards, aqueous solutions, and IS solutions were prepared on a daily basis and used immediately to avoid decomposition.

Extraction Procedure. The procedure developed for the extraction studies involved the addition of 2 mL of the extraction solvent to 1 mL of the freshly prepared 5 mg/mL aqueous standard solution (above) in a centrifuge tube which was then vortexed. Sufficient salt was added to the samples such that a small amount of solid remained after vortexing. This required approximately 1.5 g for NaCl, 1.3 g for LiCl, 2.3 g for NH_4OAc (pH 8.1), 1.5 g for KF, and 0.5 g for $NaHCO_3$ (pH 8.4). If the solution was to be acidified, 60 μL of 2.4 N HCl was added prior to the addition of solvent or salt, which adjusted the pH to approximately 1. When additions were complete, the mixture was vortexed and allowed to separate and the supernatant liquid was removed using a Pasteur pipet. Another 1-mL aliquot of the extracting solvent was added, the mixture vortexed, and the supernatant removed. Repeating this 1-mL extraction one more time gave a total extraction solvent volume of approximately 4 mL as measured in a graduated 15-mL centrifuge tube. Average recoveries of the total 4 mL of extracting solution (triplicate extractions) are shown in Table I. The recovered extraction volume was made to 10 mL with extraction solvent after 1 mL of the solution containing the internal standard in the same solvent was added.

Both of the solutions (extract and reference standard) were analyzed by GC using a 5890A Hewlett-Packard gas chromatograph with a flame ionization detector and a 15 m × 0.25 mm i.d. DB-5 fused silica capillary column with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA). Oven temperature was isothermally maintained at 95 °C with the injection port at 250 °C, detector at 275 °C, and a helium gas flow of 1 mL/min. A Model HP7673 automatic sampler with sample trays cooled to 15 °C was set to deliver triplicate 2-μL injections into a split injector (measured split ratio 100:1 ± 1%) containing a Jennings-type split line with 10% OV-1 on 8/100 Chromasorb WHP column packing as received from the manufacturer (HP 18740-60840). All GC data were collected in an HP 9000 Series 300 computer using HP 5985A GC ChemStation software.

Under these conditions of analysis, phenol gave a retention time of 1.74 min, the IS 2,6-dimethylphenol 3.19 min, catechol 5.14 min, hydroquinone 7.94 min, and resorcinol 8.58 min. Figure 1 shows the chromatogram obtained for this combination.

Table I. Average of Triplicate Recovered Volumes (Milliliters) of Extracting Solvents[a]

solvent		KF	NaCl	NH_4AcO	LiCl
		From Neutral Solution			
Et_2O	av	3.5	3.7	3.6	3.2
	sd[b]	0.1	0.1	0.1	0.1
THF	av	3.6	4.0	3.9	c
	sd	0.2	0.1	0.1	c
EtOAc	av	3.5	3.9	4.0	3.9
	sd	0.1	0.1	0.1	0.1
ACN	av	3.9	4.0	3.7	3.3
	sd	0.2	0.1	0.1	0.1
DMF	av	3.7	c	c	c
	sd	0.1	c	c	c
		From Acidified Solution[d]			
Et_2O	av	3.4	3.6	3.5	3.2
	sd	0.2	0.1	0.1	0.1
THF	av	3.6	4.0	3.9	c
	sd	0.3	0.1	0.1	c
EtOAc	av	3.6	3.9	3.9	4.0
	sd	0.1	0.1	0.1	0.1
ACN	av	3.5	3.9	3.5	3.4
	sd	0.2	0.1	0.1	0.1
DMF	av	3.9	c	c	c
	sd	0.1	c	c	c

[a] See Experimental Procedures. [b] sd, standard deviation. [c] No phase separation. [d] pH 1.

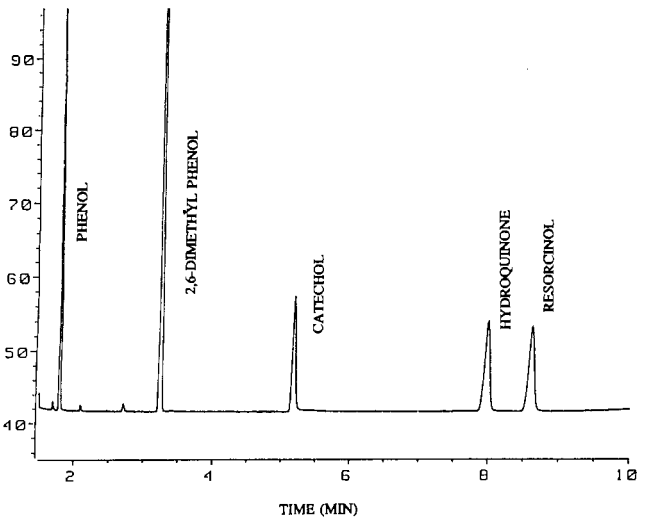

Figure 1. Typical chromatogram for the four phenols and the internal standard, 2,6-dimethylphenol. Chromatographic conditions: column, 15 m J&W DB-5, i.d., 0.25 mm; column flow, He (1 mL/min); phase thickness, 0.25 μm; injector temperature, 275 °C; oven temperature, 95 °C; detector, FID at 275 °C; split ratio, 1:100.

Response factors (RFs) for each of the above phenols in the diluted water solution were calculated using the relationship

$$RF = \left(\frac{\text{phenol area}}{\text{IS area}}\right)\left(\frac{\text{injected amt of IS}}{\text{injected amt of phenol}}\right) \quad (1)$$

where 2,6-dimethylphenol was the IS used. Using this RF, the amount of the phenols present in the extraction solvent was calculated using the following equation:

$$\text{calcd amt of phenol} = \left(\frac{\text{phenol area}}{\text{IS area}}\right)\left(\frac{\text{injected amt of IS}}{\text{RF for phenol}}\right) \quad (2)$$

The percent recovery was determined by

$$\frac{\text{calcd amt of phenol in extract}}{\text{weighed amt of phenol in solution}} \times 100 = \%\ \text{recovery} \quad (3)$$

The results of the DR and RF studies are contained in Table II.

Table II. Detector Responses (DR)[a] and Response Factors (RF)[b] for Phenols in the Solvents Studied

	compound (bp, °C)[c]									
	phenol (182 °C)		2,6-dimethylphenol[d] (203 °C)		catechol (245 °C)		resorcinol (280 °C)		hydroquinone (285 °C)	
solvent (bp, °C)	DR	RF	DR	RF	DR	RF	DR	RF	DR	RF
Et_2O (34.5 °C)	115.87	0.902	128.46		92.77	0.722	108.07	0.841	110.57	0.861
	(8.43)[e]	(0.010)	(8.81)		(6.72)	(0.018)	(8.17)	(0.020)	(8.21)	(0.017)
THF (66 °C)	138.48	0.953	145.24		85.49	0.589	123.43	0.850	114.22	0.787
	(5.68)	(0.020)	(4.35)		(5.57)	(0.037)	(3.44)	(0.011)	(3.17)	(0.009)
Et_2OAc (77.1 °C)	123.00	0.914	135.07		84.56	0.628	111.57	0.830	113.80	0.846
	(2.51)	(0.014)	(3.85)		(4.99)	(0.034)	(2.10)	(0.031)	(1.54)	(0.028)
ACN (81 °C)	120.37	0.943	127.63		62.94	0.494	113.02	0.853	104.78	0.821
	(5.58)	(0.026)	(3.33)		(6.57)	(0.053)	(5.21)	(0.025)	(4.20)	(0.025)
DMF (153 °C)	178.20	0.961	185.54		97.97	0.528	117.35	0.633	125.75	0.678
	(1.37)	(0.012)	(1.91)		(9.44)	(0.055)	(8.75)	(0.052)	(4.83)	(0.052)

[a] Conditions of GC analysis given under Experimental Procedures. Units are in area counts/ng injected. [b] From eq 1 under Experimental Procedures. RFs relative to IS 2,6-dimethylphenol. [c] Boiling points are taken from: *Merck Index*, 10th ed.; Windholz, M., Ed.; Merck & Co., Inc.: Rahway, NJ, 1983. [d] IS, internal standard. [e] Data in parentheses are standard deviations of triplicate determinations.

RESULTS AND DISCUSSION

Since the detector responses differed from solvent to solvent (Table II), phenol and its dihydric metabolites had to be studied in the pure extracting solvents to provide the quantitative data necessary for determining extraction efficiency.

The conditions of capillary column GC analysis involving split injection to avoid sample discrimination (i.e., enhanced detector responses for low-boiling solutes at the expense of the higher boiling solutes) were those recommended by Poole and Schuette (1984). This involved the following criteria: (1) The concentration of all the phenols in each solvent was very nearly the same, and the same sample volume (2 μL) was used for all samples. (2) Rapid and reproducible needle penetration was accomplished through the use of an autosampler. (3) The column GC analysis was performed isothermally at a column temperature of 95 °C with an injection port temperature of 250 °C. (4) An internal standard was used in all analyses. (5) The split ratio of 100:1 was measured and adjusted for each analysis and/or when the solvent was changed. That the conditions imposed were reasonably successful in minimizing sample discrimination is indicated by the similarity of detector responses (DRs) obtained (Table II).

It is to be noted that as the boiling point of the solvent increased, the DRs of the analytes showed some increase also, as shown in Table II and Figure 1, the exceptions being the values obtained with EtOAc and ACN. The DRs obtained with the highest boiling solvent, DMF, were the highest of any of the solvents studied, the one exception being resorcinol. The enhanced DR with this solvent is consistent with the "recondensation" concept which attributes the increase in area (DR) to the recondensation of the solvent in the cool column inlet "which creates a zone of reduced pressure in the column inlet thus sucking in further amounts of sample vapor" and "at a column temperature 50–80 °C below the boiling point of the solvent, recondensation is virtually complete". In the above case of DMF, with a bp 153 °C (Table II), an injection port temperature of 250 °C, and a column temperature of 95 °C, the conditions are within those given for recondensation (Grob and Neukom, 1982) and produced the highest DRs found in this study, the two exceptions being hydroquinone and resorcinol.

Previously, the vaporizing process in split injectors was described as being one in which the injected liquid sample generates a "thrust" or pressure increase in the injector which changes the split ratio, thus making the amount of analyte reaching the column dependent on the pressure wave developed (Grob and Neukom, 1979). Reasoning that the 2 μL of injected solution was predominantly the solvent and the volume of the 4 mm i.d. split injector liner containing 1 cm deep 80/100 Chromosorb WHP (HP Model 18740-60840) was 500 μL, the pressure developed at 250 °C (the injection port temperature) was calculated for each solvent using the equation $PV = nRT$. Using the reported densities of the solvents at 20 °C as Et_2O, 0.7134 g/cm^3, THF, 0.8892 g/cm^3, EtOAc, 0.9006 g/cm^3, ACN, 0.7822 g/cm^3, and DMF, 0.9487 g/cm^3 (Riddick and Bunger, 1990), the calculated pressures in atmospheres or, in parentheses, kilopascals were found to be 1.625 (164.653), 2.117 (214.505), 1.755 (177.825), 3.271 (331.435), and 2.28 (231.021), respectively. Plotting atmosphere developed vs DR gave Figure 2, in which the DR in DMF follows the expected increase in DR except for resorcinol. It would appear that the recondensation process operates only with DMF, while the other lower boiling solvents experience predominantly the solvent vaporization process. The higher boiling points of the analytes suggest that they should recondense on the cooler column inlet. However, as shown in Table II, only the lower boiling phenol and 2,6-dimethylphenol show significant increases in DR in progressing from Et_2O to DMF, the DRs of the dihydric phenols remaining essentially constant. This would appear to be consistent with the postulate of the higher boiling phenols undergoing a recondensation regardless of the solvent boiling point (Grob and Neukom, 1982).

After having determined DRs and RFs in the various solvents, attention was directed to the extractive effectiveness of the salting-out process of selected salts with these solvents. As shown in Table I, this recovery of water-miscible and -immiscible solvents was nearly quantitative except in the combinations of DMF/NaCl, DMF/NH_4-AcO, DMF/LiCl, and THF/LiCl, where no phase separation occurred despite the fact that undissolved salt was observed in every instance. However, that the salting-out extraction for the phenols with selected salt/solvent combinations is effective is confirmed by the data in Table III.

The solvents providing better than 92% extraction efficiency in saturated KF when the solution was acidified to pH 1 were THF and EtOAc. In saturated NaCl extractions in neutral and acidified solutions, extraction efficiencies of 97.0–100.9% were obtained with THF, Et_2O, and EtOAc, the latter in accord with previous studies (Fell and Lee, 1976), while ACN gave 92.0–103.8% of efficiency in acidified solutions only. When LiCl was used as the saturating salt, only EtOAc gave 99.6–105.1% efficiency in both neutral and acidified solutions, while Et_2O gave

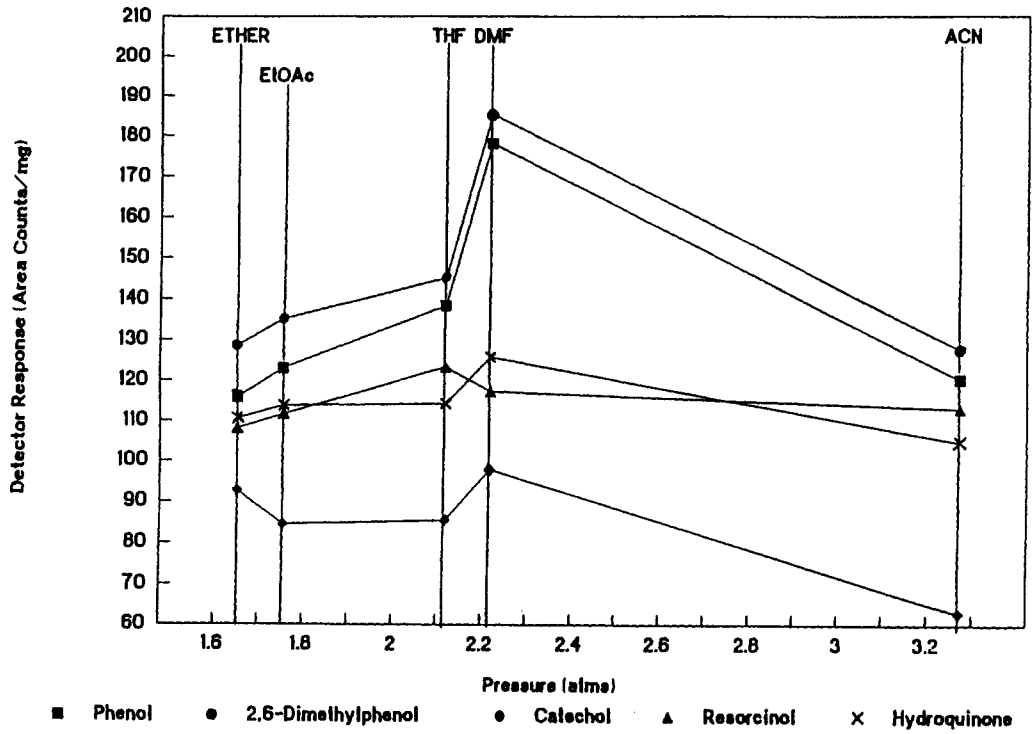

Figure 2. Plots of calculated pressures developed in the injection port vs detector responses.

98.6–101.8% in acidified solutions and 97–99% in neutral solutions. Saturating with NH_4AcO provided an extraction efficiency of only 89–99% with THF, while a saturated $NaHCO_3$ extraction gave 94.5–98.8% efficiency with EtOAc and 95.5–99.2% with Et_2O. It appeared that the three most efficient extraction solvents were Et_2O, THF and EtOAc with saturated acidified salts of KF, NaCl, LiCl. With $NaHCO_3$, THF was the only solvent providing 90% or better extraction efficiency, whereas only EtOAc was effective with $NaHCO_3$ saturation.

Although DMF exhibited excellent solubility for the phenols when standard solutions for the DR study were prepared (Table II), it showed no phase separation in saturated NaCl, LiCl, $NaHCO_3$, and NH_4AcO solutions and, when used with KF, extracted no hydroquinone from the neutral solution and only 29% from acidified solutions. The other dihydric phenols showed reduced recovery in DMF compared to the other effective extraction solvents (vide supra). No new GC peaks were observed. However, when the DMF solvent was evaporated from the extractant solution, a dark residue remained. Extracted with benzene, a GC analysis of this solution showed the presence of only phenol, catechol, and resorcinol, the hydroquinone being absent. The remaining brown material was insoluble in hexane, methylene chloride, and benzene, only partially soluble in methanol, and had a melting point >320 °C. It has been reported that oxidation of hydroquinone and catechol first yields *p*- and *o*-quinone which react further with nucleophilic reactants to produce humic acid type polymers which would have similar solvent properties (Musso, 1963).

The solvent DMF in conjunction with KF has been used in the dehydrohalogenation of 2-butyl halides (Bartsch, 1970) and 2-halogenso-1-*p*-nitrophenylpropenes (Naso and Ronzgini, 1974), in the preparation of alkyl phenyl ethers (Miller et al., 1979), in the Knoevenagel reaction (Rand et al., 1962), and in the room temperature self-condensation of some enolizable ketones (Clark and Miller, 1977a,b). The excellent yields in the fluoride ion assisted catalyzed preparation of alkyl phenyl ethers from phenols and alkyl iodides has been attributed to the H-bond formed between the protic phenol and the fluoride anion, resulting in a pseudoanionic phenolate having enhanced nucleophilicity (Miller et al., 1979; Clark and Miller, 1977a,b). In the two aprotic solvents (DMF and ACN) used here with the phenols, such bonding must be considered important. Any water present would react with the excess anhydrous KF present to give the dihydrate, minimizing any water solvation of the fluoride ion which would hinder the H-bonding with the phenols. It was found that aqueous solutions of catechol, when extracted by the DMF/KF procedure, resulted in a dark purple DMF solution with no color in the aqueous phase. A mixture of catechol and hydroquinone produced a deep purple DMF solution which, over a period of 2 h, produced a dark precipitate. It has been previously reported that the reaction of mono- and polyhydric phenols with oxygen under alkaline conditions produces dark-colored complex mixture of "poorly defined products" (Mikailovic and Cekovic, 1971).

To determine the fate of phenol, hydroquinone, catechol, and resorcinol in the DMF extract of a KF saturated aqueous solution of these phenols, triplicate DMF/KF extractions were performed on neutral aqueous solutions of (1) phenol and hydroquinone, (2) catechol and hydroquinone, (3) resorcinol and hydroquinone, and (4) hydroquinone by itself. These were analyzed for extraction efficiency as previously described. The results are shown in Table IV, and as can be seen, the DMF/KF extract of the catechol–hydroquinone combination results in total loss of hydroquinone and only 20.5% recovery of the original amount of catechol. Only 21% of the hydroquinone was recovered in the extraction procedure when the other phenols were not present.

The combinations of hydroquinone–resorcinol and hydroquinone–phenol both yield essentially the same recovery of hydroquinone (39.83% and 37.28%, respectively), while resorcinol is only 44.79% recovered and phenol is 87.21% recovered. It is apparent that whatever reactions are occurring with hydroquinone, the presence of catechol enhances the rate or, indeed, adds another reactive species consistent with the observation that these

Table III. Average Percent of Triplicate Extraction Efficiencies with Various Solvents from Saturated Salt Aqueous Solutions

	Et_2O		THF		EtOAc		ACN		DMF	
	N[a]	A[b]	N[a]	A[b]	N[a]	A[b]	N[a]	A[b]	N[a]	A[b]
Saturating Salt KF										
phenol	96.89	93.80	94.49	92.78	92.19	95.21	96.43	95.40	89.55	93.04
	(1.15)[c]	(3.12)[c]	(2.52)	(2.91)	(1.04)	(2.03)	(1.11)	(2.45)	(1.65)	(1.12)
catechol	95.03	93.75	89.87	93.39	86.39	94.02	52.67	73.79	68.47	87.18
	(3.16)	(1.56)	(5.21)	(5.14)	(3.66)	(3.54)	(9.05)	(9.16)	(3.94)	(3.03)
hydroquinone	91.77	88.27	76.16	93.57	75.13	92.22	14.62	21.86	0.00	29.09
	(1.48)	(2.48)	(4.79)	(3.85)	(2.94)	(3.48)	(1.80)	(2.29)		(11.19)
resorcinol	89.83	91.27	85.87	94.60	77.13	94.04	35.26	83.27	62.63	89.58
	(2.05)	(1.97)	(2.99)	(3.05)	(4.91)	(4.26)	(2.35)	(5.73)	(2.20)	(3.26)
Saturating Salt NaCl										
phenol	100.16	99.80	97.25	96.99	99.68	99.37	100.84	100.66	no phase separation	
	(2.78)	(1.12)	(0.81)	(1.06)	(1.45)	(1.07)	(2.16)	(2.46)		
catechol	101.58	102.54	95.97	97.41	97.05	99.36	88.53	92.44		
	(3.51)	(2.22)	(4.52)	(5.48)	(6.22)	(3.28)	(5.91)	(9.38)		
hydroquinone	102.31	101.08	99.77	100.02	100.66	100.95	101.64	103.31		
	(2.49)	(1.14)	(1.48)	(1.68)	(4.31)	(3.68)	(2.92)	(2.61)		
resorcinol	102.16	101.32	100.34	100.31	100.89	100.75	100.89	103.84		
	(2.86)	(1.18)	(1.68)	(1.37)	(4.66)	(4.16)	(1.94)	(2.46)		
Saturating Salt LiCl										
phenol	98.40	98.64	no phase separation		99.57	99.57	97.74	97.44	no phase separation	
	(1.49)	(2.87)			(0.40)	(0.97)	(1.09)	(1.07)		
catechol	99.24	101.81			100.04	105.11	97.56	104.29		
	(2.55)	(4.25)			(1.64)	(2.64)	(5.86)	(6.29)		
hydroquinone	96.94	99.02			101.73	103.16	88.18	86.28		
	(1.94)	(2.28)			(1.02)	(1.75)	(3.30)	(1.75)		
resorcinol	98.78	99.70			100.51	101.07	97.05	94.01		
	(1.81)	(2.30)			(0.45)	(1.89)	(3.25)	(1.33)		
Saturating Salt $NaHCO_3$										
phenol	99.16		no phase separation		98.86		no phase separation		no phase separation	
	(0.92)				(2.23)					
catechol	97.42				96.81					
	(2.21)				(1.25)					
hydroquinone	95.53				94.49					
	(0.82)				(1.12)					
resorcinol	97.29				95.23					
	(1.32)				(1.12)					
Saturating Salt NH_4OAc										
phenol	97.09		98.59		97.61		94.56		no phase separation	
	(0.49)		(2.47)		(0.67)		(0.52)			
catechol	43.54		88.29		62.76		25.29			
	(3.27)		(6.40)		(1.90)		(2.86)			
hydroquinone	24.28		89.04		49.23		31.23			
	(1.71)		(2.09)		(0.94)		(1.69)			
resorcinol	34.71		94.60		58.57		37.19			
	(2.14)		(5.47)		(1.35)		(1.02)			

[a] Neutral solution. [b] Acidic solution, pH 1. [c] Values in parentheses are standard deviations of triplicate determinations.

Table IV. Percent Extraction Efficiencies of Triplicate DMF/KF Extractions of Hydroquinone and Hydroquinone/Other Phenol Mixtures[a]

DMF extract color	phenol	catechol	hydroquinone	resorcinol
yellow-brown	87.21		37.28	
	(5.31)		(3.41)	
dark brown		20.50	0.00	
		(1.79)	0.00	
yellow-brown			39.83	44.79
			(3.20)	(5.41)
yellow-brown			21.08	
			(1.38)	

[a] Values in parentheses are the standard deviations of triplicate determinations.

dihydric phenols show enhanced susceptibility to oxidation (Bartsch, 1970).

Another solvent, ACN, produced different unexpected results when used to extract KF saturated solutions. As described under Experimental Procedures, the combined 4 mL of the ACN extract had added to it the IS solutions and the solution was made to volume and analyzed by GC. Hydroquinone was reduced 70–85% relative to the reference solution, and, for the first time, quinone was observed (identified by GC enrichment and GC/MS) and showed a concomitant increase as the hydroquinone decreased. The appearance of quinone was observed only in the KF/ACN extraction combination. When this same extraction was performed on the acidified aqueous solution, the amount of quinone and hydroquinone obtained was increased. No other saturating salt investigated with ACN as the extracting solvent produced quinone, emphasizing the uniqueness of the ACN/KF combination. No comparable oxidation involving ACN/KF has been previously reported.

To determine if dissolved air in the water or ACN used in the extraction was the oxidation source, these solvents were sparged with helium for 24 h and then placed in a nitrogen-filled Atmos Bag (Aldrich), which contained a vortex. All volumetrics were filled with nitrogen before being placed in the bag. The triplicate samples of phenols were weighed out in the Atmos Bag and made to volume (see Experimental Procedures) with the sparged water as was the IS, making the latter to volume with the sparged

Table V. Amount of Quinone Deriving from Hydroquinone in Single ACN/KF Extractions

aqueous medium	A, hydroquinone av ng found	B, quinone av ng found	A + B in hydroquinone equiv	orig amt of hydroquinone	% recovery of hydroquinone
neutral	0.772	1.510	2.282	5.059	45.11
	0.647	0.952	1.599	5.055	31.64
	0.794	0.764	1.558	5.023	31.03
acidic	1.078	3.189	4.217	5.059	84.34
	1.111	3.315	4.426	5.055	87.57
	1.120	2.652	3.772	5.023	75.10

Table VI. Calibration of Duplicate Water Standards with Benzene as Internal Standard

amt of benzene, μL	amt of H_2O, μL	amt ratio
10.00	10.00	1.00
10.00	20.00	2.00
10.00	30.00	3.0

integrated ^1H benzene	av integrated ^1H water	av response ratio
6.00	11.19	1.87
6.00	23.92	3.99
6.00	34.51	5.75

Table VII. Percent Water Remaining in ACN following KF Saturation of 2:1 ACN/Water Using Benzene as Internal Standard

extn	response: benzene	response: water	response: ratio	amt of water, μL	% water remaining
1	6.00	4.20	0.699	3.69	0.72
2	6.00	4.77	0.795	4.18	0.82
3	6.00	4.97	0.828	4.36	0.85

ACN. The bag was evacuated and filled with nitrogen three times, after which the extraction process with sparged ACN was performed as before and the extracts were analyzed by GC.

The results in terms of quinone produced and hydroquinone consumed are shown in Table V, and it is to be noted that the sum of the remaining hydroquinone and quinone does not equal the initial hydroquinone concentration. This would suggest that other unknown reactions are occurring.

A search of the literature produced no information as to analogous reactions. The uniqueness of the ACN/KF combination in the production of quinone under anaerobic conditions eliminated the possibility of hydroquinone oxidation by molecular oxygen as described by Musso and Döpp (1967). Research is underway to further investigate this unusual reaction.

During these extraction studies, it was noted (in parallel NMR studies) that KF was particularly effective in removing water from the extracting solvent. It was decided to determine the solvent drying potential of anhydrous KF.

^1H NMR was used to determine the percent water left in acetonitrile after extraction from water using KF (anhydrous) to salt out and dry the acetonitrile, incorporating an internal standard (benzene). Integration was used to determine the number of protons represented by a peak relative to the other peaks in the spectrum. Thus, if two compounds were present in solution in known concentrations, then their relative concentrations would give rise to a value for their relative integration values. The relative concentrations of internal standard benzene (B) to water (A) would result in an amount ratio (concentration of compound A/concentration of compound B) and the relative integrations in a response ratio (integration of compound A/integration of compound B). With this information, one could calibrate the NMR using a set amount of compound B (benzene) to various amounts of compound A (water).

Benzene yields only a sharp singlet at 7.4 ppm, far removed from the singlet due to water which appears in the region 2–3.5 ppm, depending on concentration. Three standards were prepared by adding 10 μL of benzene to separate 0.5-mL aliquots of ACN-d_3. To the first standard was added 10 μL of water, to the second 20 μL, and to the third 30 μL. These were all scanned on a General Electric 300 MHz Fourier transform NMR (32 scans). The integration for the benzene peak was fixed at 6.00 protons, and the integration for water is relative to this value. Table VI shows the results of a duplicate study and contains the amounts of each compound used, the amount ratios, the integration values for each compound, the response ratios, and the regression results of the amount ratio versus the response ratio. The x coefficient and the constant intercept value from the regression will allow determination of unknown concentrations of water in acetonitrile from the equation $y = ax + b$. In this regression

$$y = \frac{\text{integrated H's for } H_2O}{\text{integrated H's for } C_6H_6 \text{ set at } 6}$$

$$x = \frac{\text{amt of } H_2O}{\text{amt of } C_6H_6}$$

and b is the intercept. The R^2 value for the study was found to be 0.9972.

Triplicate extractions were performed by combining 0.5 mL of HPLC water with 1 mL of ACN-d_3 and adding ca. 1.5 g of anhydrous KF. A 0.5-mL aliquot of each extract had added to it 10 μL of benzene. The NMR was scanned as above, and using the relative integrations and the calibration in Table VI, the percent water remaining was determined in each sample (Table VII). The calculated 99.1% drying efficiency of anhydrous KF would indicate the salt to be an effective drying agent.

The fact that anhydrous KF is an effective drying agent lends it to be useful in a unique method of extraction in which water-miscible solvents may be extracted and dried, quantitatively, in a one-step process. For this reason the combination of THF/KF to extract phenols from acidified, aqueous media was chosen by the authors for use in the extraction of phenols from aqueous biological samples in which the KF drying process is necessary for the conditions of a fluorobenzylation derivative reaction (Jones et al., 1993).

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