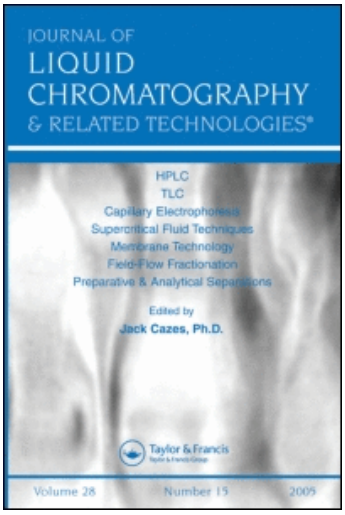

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ANALYSIS OF PENTACHLOROPHENOL IN DRINKING WATER AND HUMAN URINE BY HPLC WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

High performance liquid chromatography with electrochemical detection (HPLC-EC) was applied to the analysis of pentachlorophenol (PCP) in drinking water and human urine. Lower detection limits for PCP in drinking water was approximately 1-5 ppb while the chlorinated phenol could be detected in human urine at about 10 ppb concentration. Analyses of PCP in drinking water can be made with no sample pretreatments, while analyses involving human urine required acid hydrolysis, absorption on an anion exchange resin, and desorption with methanol. In each case dramatic savings in analysis times and increases in sample through-put are realized using analysis based on HPLC-EC rather than the more traditional procedures relying on gas chromatography with electron capture detection. Method sensitivities were approximately equivalent to methods using gas chromatography.

INTRODUCTION

Pentachlorophenol is a widely used pesticide and preservative. Its use is especially prevalent in the southern portions of this country, particularly in the hot, high humidity regions of the Gulf and Atlantic coasts. It is employed throughout these areas for treating wood products to prevent attack by microorganisms. Because of its wide use PCP has become a ubiquitous environmental pollutant, and has been found to be widely present in human urine in this country at low to mid ppb (ng/ml) concentrations (1). Accumulation of PCP in the human population of this country presumably results from contact with wood products such as paper and cardboard, ingestion of contaminated food-stuffs or water supplies, and contact with materials used in construction of dwellings for human habitation. Like many halogenated organic compounds PCP is toxic and readily accumulates in high lipid-content animal tissues.

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Traditional protocol (1-3) for analysis of PCP and related chlorophenols in human urine is based on gas chromatography with electron capture detection (GC-EC), a technique not notably compatible with the aqueous media of drinking water and human urine. The initial step in the protocol for urine consists of acid hydrolysis to free PCP from its glucose or sulfate conjugates. Free phenol is extracted with benzene (a suspected carcinogen), methylated with diazomethane (a toxic explosive suspected carcinogen) and the resulting fraction analyzed by gas chromatography or purified by column chromatography and then subjected to analysis. These wet chemical manipulations may require several hours to effect. Analysis of municipal drinking water could be accomplished using the above procedure with elimination of the acid hydrolysis step.

Analysis of aqueous media for PCP was approached in the present study by substituting HPLC-EC for GC-EC. Since HPLC-EC typically employs reversed phase columns with mobile phases of aqueous buffers mixed with varying amounts of water-miscible organic solvents to control retention the technique is compatible with urine or other aqueous samples. In addition, electrochemical detection is highly sensitive to phenols. Thus, it seemed reasonable that an analytical method based on HPLC-EC might greatly reduce sample preparation time and markedly increase sample through-put.

Other researchers have appreciated the promise held by HPLC for analysis of biological materials for chlorinated phenols. Lores, Edgerton, and Moseman used HPLC-EC to confirm the presence of a large number of chlorinated phenols in human urine after primary analysis by gas chromatography(4). Earlier these researchers had reported recoveries of chlorinated phenols (10 bbp) from fortified urine of from 79 to 88% after adsorption and desorption from XAD-4 resin into 10% 2-propanol in hexane (5). During the latter study GC-EC was employed as the analytical method.

In this study we surveyed recoveries and background interferences arising during isolation by resin adsorption and desorption of pentachlorophenol in fortified drinking water and urine and during isolation of PCP from these matrices by acid-base solvent extractions.

MATERIALS AND METHODS

High Performance Liquid Chromatography

HPLC experiments were carried out using several modular liquid chromatographs assembled in this laboratory. Mobile phase delivery systems used single piston minipumps (Laboratory Data Control, Riveria Beach, FL) with pulse dampners (Handy and Harman Tube Co., Norristown, PA) and pressure gauges (Alltech Associates, Deerfield, IL). The electrochemical detectors consisted of glassy carbon electrodes maintained at 1.0 V vs. Ag/AgCl reference electrodes by a commercial potentiostat (Bioanalytical Systems, West Lafayette, IN), a model 174 Polarographic Analyzer (Princeton Applied Research, Princeton, NJ), or a potentiostat constructed in the laboratory with a circuit similar to one described earlier (6). Chromatography columns were 4.6 mm i.d. by 250 mm stainless steel obtained commercially or packed in this laboratory. Spherisorb ODS, 5 μm, (Alltech Associates), laboratory prepared and packed trimethylsilyl reversed phase on 8 μm sperical silica gel, and cyanopropylsilyl bonded phase on 10 μm silica (Alltech Associates) gave approximately equivalent retention times for PCP when mobile phases of 0.1 M $NH_4H_2PO_4$ with 70, 50, and 30% acetonitrile, respectively, were pumped through the columns at 1 ml/min.

Sample introduction was by valve-loop injection using a 7000 psi valve (Valco Instruments, Houston, TX).

Acid Hydrolysis Of Urine

Urine samples were hydrolyzed according to a published procedure (4) or by the use of sulfuric acid at 100°C (3 ml conc. H_2SO_4, 5 ml urine, 30 min). Hydrolyses were conducted in culture tubes with teflon-faced screw caps.

Solvent Extraction And Resin Adsorption Of PCP From Hydrolyzed Urine

Solvent extractions of PCP from urine (and from distilled water) was performed according to the method outline in Fig. 1. Extractions were carried out in screw-capped culture tubes with as few sample transfers as possible.

Anion exchange resins (AG1-X2, and AG1-X8) and BioBeads S-X8 were purchased from BioRad Laboratories, Richmond, CA and Amberlite XAD-2 and Tenax from Alltech Associates. The resins were washed with methanol (3 X 5 ml) and with distilled water (3 X 5 ml) after being packed into disposable Pasteur pipets.

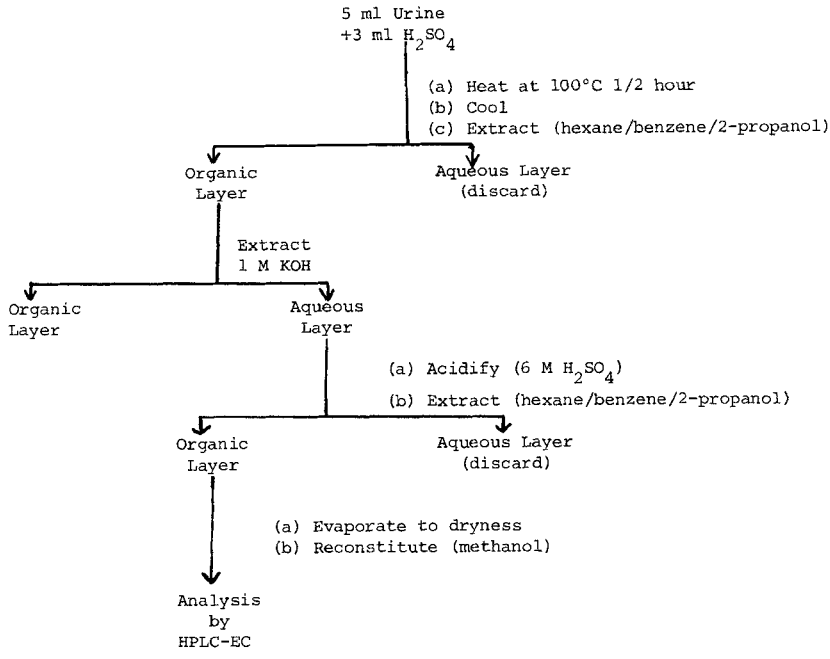

Figure 1. Flow chart for Hydrolysis and Extraction of Urine Samples.

The resin columns (ca. 3 cm bed height) were used to adsorb 5 ml samples of urine hydrosylate and washed with deionized water (3 X 5 ml). Adsorbed material was desorbed with 5 ml of methanol and the elute was analyzed directly by HPLC-EC. Recoveries were estimated by comparison with PCP standards of appropriate concentration.

RESULTS

To test the utility of HPLC-EC for analysis of PCP in aqueous solutions we first ran a series of drinking water samples to which varying amounts of the chlorinated phenol had been added. The water samples were injected directly with no sample clean-up. Chromatograms from analysis of 20 µl of drinking water spiked with 20 ppb PCP (A) and drinking water without added phenol (B) are shown in Fig. 2. These results indicate that analysis of about 2 ppb PCP in drinking water, is possible with the method as described without any pre-analytical sample manipulation. Furthermore, limit of detection could be easily increased by

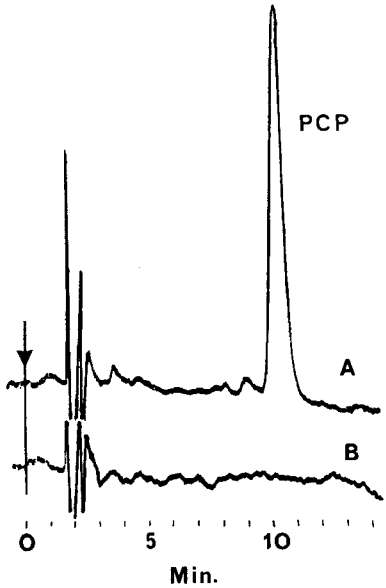

Figure 2. HPLC-EC analysis of (A) 20 ppb PCP in municipal drinking water and (b) water without added PCP. Conditions: 50 µl injections; sensitivity 10 nAFS; cyanopropyl reversed phase column; 25% CH_3CN in 0.1 M $NH_4H_2PO_4$, pH 4.0; 1.2 ml/min.

using shorter chromatography columns, chromatography columns with smaller internal diameters, larger sample sizes, or a combination of these or other modifications. Mid-ppt measurements should be attainable.

We next turned to human urine as a sample matrix. While urine can be injected directly onto the chromatography columns, the many protein constituents of the materials will cause rapid degradation of expensive columns. Furthermore the many electroactive constituents will lead to high backgrounds and decreased sensitivity, and since a substantial portion of PCP is present in urine as conjugates, much of the material will not be detected.

We first used an acid hydrolysis step followed by an acid/base/solvent extraction procedure as outlined in Fig. 1. The resulting chromatograms from HPLC-EC analysis of human urine (A) and human urine which had been spiked with 100 ppb PCP (B) are shown in Fig. 3. While PCP is easily identified and measured at this level, there are considerable electroactive substances isolated during

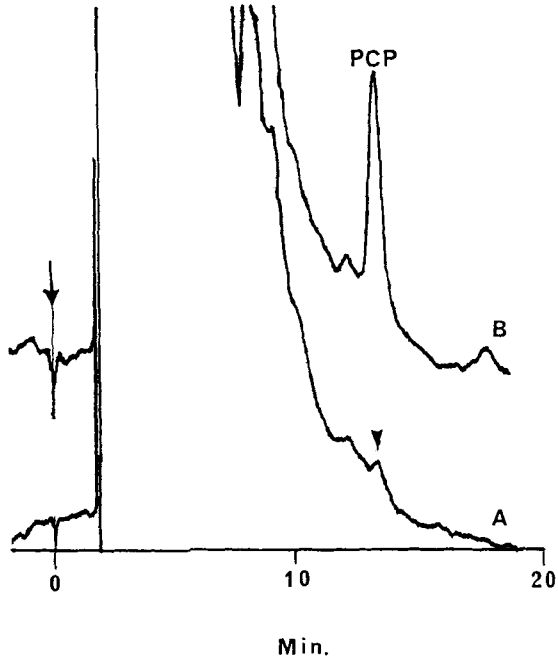

Figure 3. HPLC-EC analysis of (A) hydrolyzed human extract and (B) extract of hydrolyzed human urine fortified with 100 ppb PCP. Conditions: 20 µl injections; sensitivity, 50 nAFS; trimethylsilyl reversed phase column; 50% CH_3CN in 0.1 M $NH_4H_2PO_4$, pH 4.0; 1.0 ml/min.

work-up. These substances prevent substantial increases in sensitivity and appear to cause fouling of the working electrode. Sample work-up requires considerable time and effort.

The utility of resin adsorption of acid hydrolyzed fortified urine was then tested. Urine samples fortified with varying amounts of PCP were hydrolyzed using the procedure outlined in Fig. 1, or as previously described (4). The urine was passed through short resin columns as described in the methods section, the columns were washed with water and the PCP was then desorbed with methanol. The methanolic solutions were analyzed directly by HPLC-EC. A representative chromatogram from the analysis of human urine fortified with 100 ppb is given in Fig. 4. The resin employed in obtaining this chromatogram was AG1-X2 anion exchange resin which gave the best combination of high recovery and

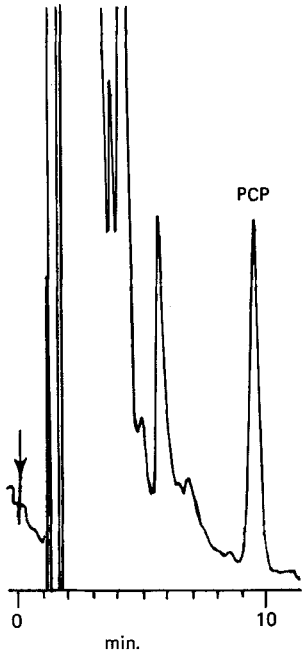

Figure 4. HPLC-EC analysis of PCP (100 ppb) isolated from fortified human urine by adsorption on AG1-X2 resin as described in the text. Conditions: 50 µl injection; sensitivity 50 nAFS; Spherisorb ODS column; 70% CH_3CN in 0.1 M $NH_4H_2PO_4$, pH 4.0; 1.0 ml/min.

relative freedom from interfering materials. Recoveries from fortified urine (200 ppb level) were 108 ± 9% while recoveries from distilled water (200 ppb level) processed according to this method were 93 ± 3% (mean ± sample standard deviation of ten or more samples).

DISCUSSION

The combination of analyte isolation by anion exchange resin adsorption and HPLC-EC analysis allows monitoring of human urine for PCP at low ppb levels. The pre-analytical sample manipulations are simple, they involve few transfers from one container to another, and employ a minimum of reagents. There are no steps involving evaporation of organic solvents, which is both expensive and time consuming, and derivatization is not required. Because of the simplicity and speed of the method and its high sensitivity it is a logical candidate for monitoring PCP contamination in large populations.

ACKNOWLEDGMENT

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