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APPLICATION OF A DIRECT AQUEOUS ACETYLATION TECHNIQUE TO THE GAS CHROMATOGRAPHIC QUANTITATION OF NITROPHENOLS AND 1-NAPHTHOL IN ENVIRONMENTAL WATER SAMPLES

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SUMMARY

An improved method for the determination of low levels of nitrophenols in aqueous samples has been developed. The method is based on the gas chromatographic analysis of phenols as acetate derivatives which have been prepared directly in water. Recoveries of 97% or greater were obtained from aqueous solutions containing nitrophenols in concentrations ranging from 25 to 100 μg/l. Concentrations as low as 1 μg/l were easily detected. Nitrophenols appear to exhibit some resistance to degradation by microorganisms indigenous to the Athabasca River. Water samples to which 139 μg/l each of *o*- and *p*-nitrophenol were added did not show evidence of microbial metabolism over a two week interval. Under identical conditions 100 μg/l of *m*-cresol was metabolized within a 3-day period.

INTRODUCTION

Carbaryl and methyl and ethyl parathion (Fig. 1) are among the most commonly used biodegradable pesticides in North America[1,2]. They are degraded in both man and environmental soil or water systems to simple phenols; carbaryl is converted to 1-naphthol[2–5] and parathions to *p*-nitrophenol[1,6,7]. Other sources of the nitrophenols

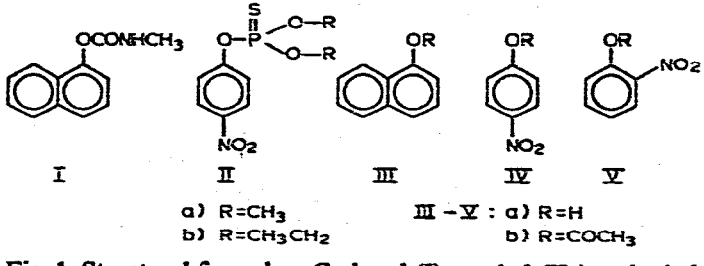

Fig. 1. Structural formulas: Carbaryl (I), methyl (IIa) and ethyl (IIb) parathion, 1-naphthol (IIIa), 1-naphthyl acetate (III b), *p*-nitrophenol (IVa), *p*-nitrophenyl acetate (IVb), *o*-nitrophenol (Va) and *o*-nitrophenyl acetate (Vb).

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and 1-naphthol include dye manufacturing industries[8] and petrochemical refineries[7,9]. Sensitive, specific analytical procedures are required to measure trace amounts of these phenols because of their toxicity[10–12] and the potential utility of *p*-nitrophenol and 1-naphthol as indicators of contamination by the pesticides parathion and carbaryl[1,2,13].

Nitrophenols have been analyzed by gas chromatography (GC) after conversion to the corresponding ethyl ethers with diazoethane[13,14]. Complex reaction schemes involving methylation of the phenolic group, reduction of the nitro constituent and subsequent trifluoroacetylation of the resulting methoxyaniline have been reported[12]. Nitrophenols may also be quantitated as heptafluorobutyryl derivatives following reduction of the nitro group[7]. Similar heptafluorobutyryl derivatization methods have been applied to the quantitation of 1-naphthol[2]. Phenols and phenol-generating pesticides have also been quantitated as 2,6-dinitro-4-trifluoromethyl phenyl ethers[15]. Even though these analytical methods are capable of detecting concentrations in the 20–100 μg/l range, the procedures used are time consuming and require a number of sequential extraction and derivatization steps. For example, most acetylation procedures currently in use require a preliminary extraction of the phenol from aqueous solution into diethyl ether[16] or benzene[17] followed by a back extraction from the organic solvent into a basic aqueous solution. We have found[18] that extraction of aqueous solutions by organic solvents or adsorption and elution from macroreticular resins usually resulted in a low recovery of phenolic compounds from these aqueous solutions. In contrast, trace amounts of some methyl- and chlorophenols could be directly acetylated in water and the resulting esters quantitatively extracted and analyzed by GC. Using this procedure, microgram amounts of phenols in large volumes of water can be easily and rapidly quantitated. The aqueous acetylation method has now been applied for the quantitation of microgram quantities of *o*-nitrophenol, *p*-nitrophenol and 1-naphthol in spiked water samples.

EXPERIMENTAL

Apparatus

The gas chromatograph was a Hewlett-Packard Model 5702A equipped with a flame-ionization detector coupled to a Model 3380A integrator. Mass spectra were recorded using a combined Hewlett-Packard Model 5710A gas chromatograph/Model 5981A mass spectrometer/Model 5934A data system. The Kuderna Danish evaporator was obtained from Ace Glass (Vineland, NH, U.S.A.). The New Brunswick Model G2 Shaker was obtained from New Brunswick Scientific (New Brunswick, NJ, U.S.A.).

Chromatographic conditions

For both GC and combined GC–mass spectrometry (MS) the glass column (1.26 m × 4 mm) used was packed with 5% OV-101 on Chromosorb W (80–100 mesh). The operating conditions for both GC and GC–MS were: column temperature, 100–220°C (8°C/min); detector, 250°C; injector, 250°C; helium flow-rate, 60 ml/min.

Reagents

Sodium bicarbonate, acetic anhydride, methylene chloride, 1-naphthol, *p*-

nitrophenol and *m*-cresol were obtained from Fisher Scientific Company (Fair Lawn, NJ, U.S.A.) while *o*-nitrophenol was obtained from Eastman-Kodak Company (Rochester, NY, U.S.A.). Methylene chloride was distilled before use.

Preparation of test solutions

Standard stock solutions of *o*- and *p*-nitrophenol, and 1-naphthol (0.1 m*M*) were prepared in 95% ethanol and stored in glass-stoppered bottles at 4°C. Prepared aqueous solutions (250 ml) containing 57.6 μg/l of 1-naphthol and from 1.0–100 μg/l of *o*- and *p*-nitrophenol were acetylated by the addition of 500 μl acetic anhydride and 10 g of $NaHCO_3$ as previously described[18]. The water was extracted with three 10 ml volumes of methylene chloride and the combined extracts were concentrated using a Kuderna Danish evaporator[19] to a volume of approximately 3.0 ml. The concentrator tube was then placed in a beaker of warm water (70–80°C) and the volume was reduced to 20–30 μl by passing a gentle stream of nitrogen over the solution. A 1-μl sample was injected into the gas chromatograph.

Extraction and derivatization –Athabasca River water

Sodium bicarbonate (30 g) and acetic anhydride (2 ml) were added to a 750-ml sample of Athabasca River water spiked with 14.4 μg (0.1 μmole) of 1-naphthol internal standard. A 1-l separatory funnel was used as the reaction vessel. When the reaction was complete, the solution was extracted with three volumes of methylene chloride (50, 25, 25 ml). The combined extract was concentrated as described above for test solutions, and a 1-μl sample was analyzed by GC.

Stability of o- and p-nitrophenol and m-cresol in Athabasca River water

Athabasca River water samples were collected at three different sites. A portion of each (400 ml) was spiked with 139 μg/l of each *o*- and *p*-nitrophenol, and 100 μg/l of *m*-cresol. The samples, placed in 1-l Erlenmeyer flasks, were stoppered with sponge and shaken at 20°C using a New Brunswick Model G2 Shaker. Aliquots (50 ml) were removed at intervals over a 2-week period and frozen until analyzed. For analysis, 0.5 ml of the 0.1 m*M* 1-naphthol solution (internal standard), 5 g $NaHCO_3$ and 500 μl acetic anhydride were added to each aliquot. After the acetylation reaction was complete, the aqueous solution was extracted and the extract concentrated and analyzed by GC as described above.

Mass spectra

The phenols and their acetate derivatives (Fig. 1, III–V), were positively identified by GC–MS *m/e* (% relative abundance): IIIa, 144(100), 116(53), 115(99), 89(11); IVa, 139(100), 123(6), 109(55), 65(21), 63(8); Va, 139(100), 109(33), 81(21), 65(17), 63(12); IIIb, 186(21), 144(100), 116(35), 115(65), 89(9), 43(2); IVb, 181(74), 139(78), 123(25), 109(100), 93(20), 65(18), 64(15), 63(23), 43(44); Vb, 181(13), 139(100), 123(4), 109(25), 81(10), 65(6), 63(12), 43(25).

RESULTS AND DISCUSSION

Carbaryl and the parathion pesticides (Fig. 1), as well as other industrial chemicals and dyes may contribute to the concentration of the nitrophenols and

1-naphthol found in river waters and human urine. Both *o*- and *p*-nitrophenol are listed as priority pollutants by the World Health Organization[11] and the U.S. Environmental Protection Agency[20]. The need for monitoring human exposure to low levels of biodegradable pesticides initially prompted the development of a number of analytical procedures[1,6,7,14]. Nitrophenols have been identified in human urine at concentrations of 12–26 and 18–67 ng/ml in the general U.S.A. population and parathion-exposed subjects, respectively[7].

The International Council on Environmental Pollutants[21] has prepared a list of reference water pollutants which are used to compare new analytical procedures with existing methodology. *p*-Nitrophenol and 1-naphthol are included in the list of pollutants for which present techniques are considered inadequate. The existing procedures have three potential disadvantages. They are time consuming; they require that the phenols be removed from the aqueous solutions by extraction with an organic solvent; and although most procedures are suited to the quantitation of trace amounts of phenols in small volumes of urine they are not readily adaptable to the analysis of microgram quantities of phenolic compounds in large volumes (up to 1 l) of aqueous solution.

Direct acetylation of an alkaline ($NaHCO_3$) aqueous solution by means of acetic anhydride completely converted the trace amounts of added *o*- and *p*-nitrophenol and 1-naphthol to their respective acetates. (Fig. 1, IIIb–Vb). Illustrated in Fig. 2 are the differences in retention times of the original phenols and the corresponding acetylated derivatives when chromatographed on an OV-101 column. The identities of the compounds giving rise to each peak in Fig. 2 were confirmed by MS. The major fragment ions in each spectrum were consistent with literature reports on the fragmentation of phenols and aromatic nitro compounds[22].

Acetylation of phenols proceeds rapidly to completion at room temperature

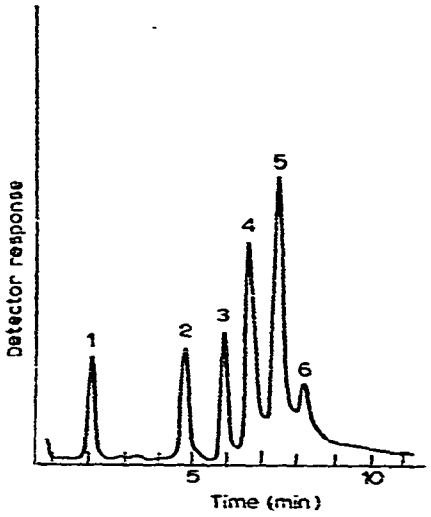

Fig. 2. GC–MS total ion trace of phenols and their acetate derivatives. Peaks: 1 = *o*-nitrophenol, 2 = *o*-nitrophenyl acetate, 3 = *p*-nitrophenyl acetate, 4 = 1-naphthol, 5 = 1-naphthyl acetate, 6 = *p*-nitrophenol. GC–MS conditions are given in the text.

and the derivatives, once extracted into methylene chloride, can be stored for at least one week at 0°C with virtually no decomposition. The gas chromatogram of *o*- and *p*-nitrophenyl acetate and 1-naphthyl acetate (Fig. 3) was obtained by adding known amounts (27.8, 27.8 and 14.4 μg) of the respective phenols to 250 ml of distilled water and acetylating these phenols directly in the aqueous solution. The conversion was quantitative. Only the acetate esters were detected by GC; peaks corresponding to the underivatized phenols were absent from the trace.

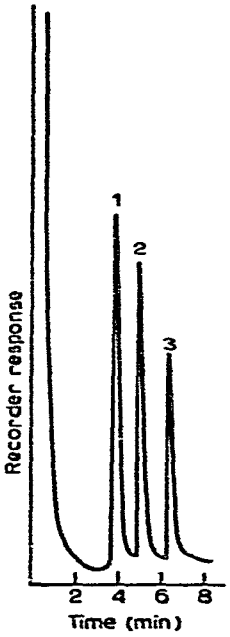

Fig. 3. GC separation of phenolic acetate derivatives prepared directly in aqueous solution. The concentration of the original phenol (μg/l) in the 250 ml water samples extracted is given in parentheses. Peaks: 1 = *o*-nitrophenyl acetate (111.2 μg/l); 2 = *p*-nitrophenyl acetate (111.2 μg/l); 3 = 1-naphthyl acetate (57.6 μg/l). The GC conditions are described in the text.

Calibration curves (Fig. 4A and B) were similarly obtained by GC analysis of methylene chloride extracts of acetylated aqueous solutions containing various quantities of *o*- and *p*-nitrophenol and the same quantity of internal standard (1-naphthol). When solutions containing 25 to 100 μg/l of *o*- and *p*-nitrophenol and 55.6 μg/l 1-naphthol were assayed by this procedure, recoveries of 97% or greater were repeatedly obtained. Detection of all three compounds at concentrations as low as 1 μg/l (Fig. 5) was possible using a flame-ionization detector. This compares favorably with other procedures for the detection of *p*-nitrophenol[14] and 1-naphthol[2] using electron-capture detection.

Athabasca River samples were examined in order to determine whether the technique of acetylating phenols in aqueous solution could be applied to natural water samples which contained a variety of organic constituents. When 750 ml of Athabasca River water spiked with 13.9 μg (0.1 μmoles) of 1-naphthol (internal standard) was

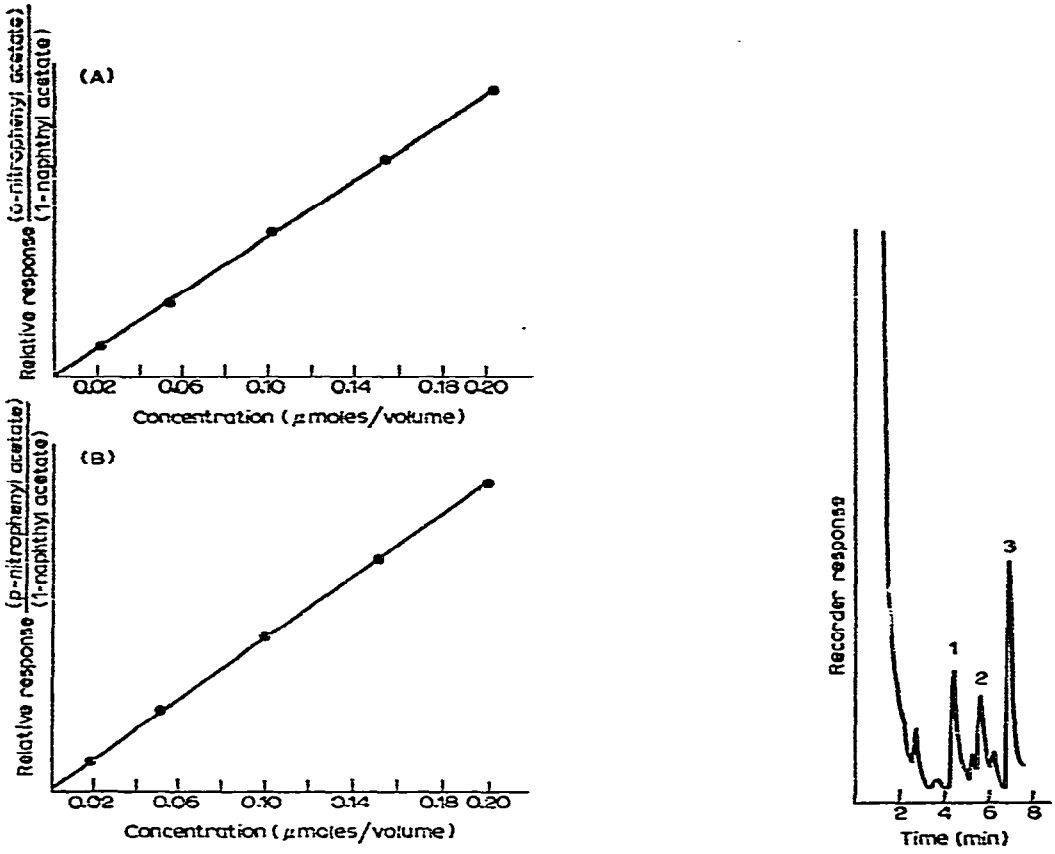

Fig. 4. Calibration graphs for the acetate derivatives of (A) *o*-nitrophenol and (B) *p*-nitrophenol in the concentration range 2.78–27.8 μg in 250 ml distilled water. The GC conditions and derivatization procedure are described in the text.

Fig. 5. A flame-ionization detector could easily detect as little as 1 μg/l each of *o*- and *p*-nitrophenol and 1-naphthol in a 500 ml water sample following aqueous acetylation. Peaks: 1 = *o*-nitrophenyl acetate, 2 = *p*-nitrophenyl acetate, 3 = 1-naphthyl acetate.

treated with acetic anhydride, peaks corresponding to the retention times of acetylated *o*- and *p*-nitrophenol were not detected. No phenolic compounds were detectable by the GC–MS analytical procedure. If the Athabasca River sample contained phenolic constituents, they were present at concentrations well below the U.S. Environmental Protection Agency's[23] upper limit of 250 and 100 μg/l for *o*- and *p*-nitrophenol, respectively, in drinking water. The U.S.S.R.[11] has introduced much more stringent guidelines for phenolic content of drinking water. Based on sanitary and toxicological grounds concentration limits of 100, 60 and 20 μg/l have been set for 1-naphthol, and *o*- and *p*-nitrophenol, respectively. Samples of water containing phenolics in excess of these USSR limits could be easily and rapidly identified using the aqueous acetylation procedure described here.

A recent review of literature reports on phenolic compounds in water[23] con-

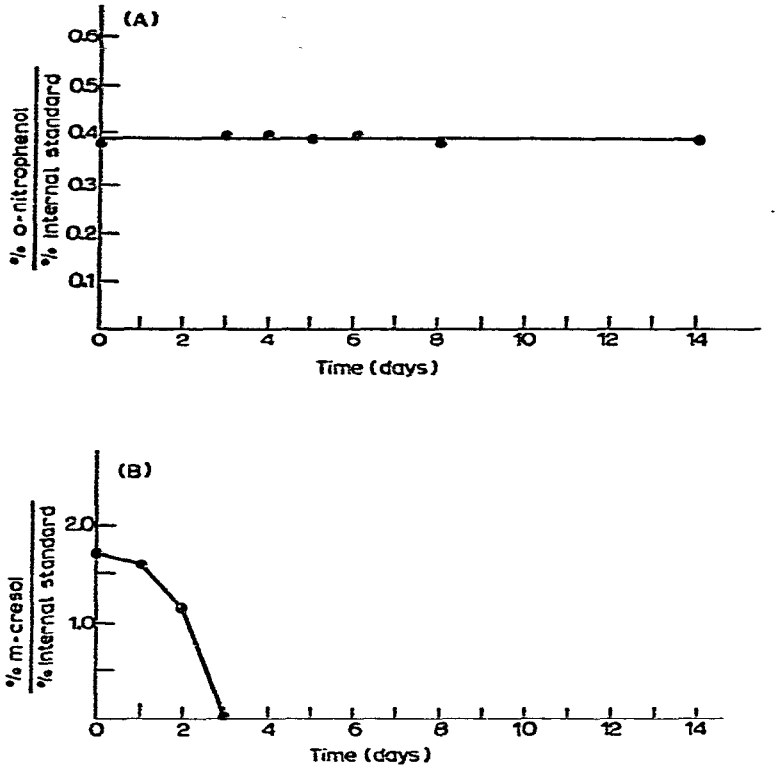

Fig. 6. The metabolism of (A) *o*-nitrophenol and (B) *m*-cresol added to Athabasca River water at concentrations of 139 and 100 μg/l, respectively. The amount of original phenol remaining was calculated using internal standard addition.

tains few references to the fate of phenols in aqueous ecosystems. In the present study when 139 μg/l each of *o*- and *p*-nitrophenol were added to Athabasca River water, the concentration of *o*-nitrophenol (Fig. 6A) and *p*-nitrophenol remained virtually unchanged over the 2-week sampling period whereas the concentration of *m*-cresol (Fig. 6B) rapidly declined within 3 days.

The persistence of organic compounds in water is very dependent on the aquatic system and its indigenous microflora. In marine water samples[24] carbaryl completely disappeared after a 17 day incubation at 20°C with 43% conversion to 1-naphthol. 1-Naphthol persisted in mud for 2 to 6 weeks[24] and a stable precipitate of unknown structure (mol.wt. 454) was thought to be responsible for the toxic effects observed in carbaryl treated areas[5]. 1-Naphthol is as toxic to a number of aquatic species[19] as the original pesticide carbaryl. The stability of phenolic pesticide metabolites is therefore important not only because these compounds are markers for monitoring pesticide contamination but also because of their inherent toxicity.

CONCLUSION

o- and *p*-nitrophenol and 1-naphthol can be rapidly detected and quantitatively analyzed in water samples at levels well below the limits set by regulatory agencies.

This is accomplished by direct acetylation of the aqueous solution followed by extraction of the resulting phenolic acetates and examination of the extract by GC. Both *o*- and *p*-nitrophenols are resistant to degradation by the natural microflora present in the Athabasca River while *m*-cresol undergoes rapid metabolism.

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