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Note

Determination of 4-aminophenol in water by high-performance liquid chromatography with fluorescence detection

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4-Aminophenol is a metabolite of the insecticide parathion as illustrated in Fig. 1[1]. Aminoparathion, the major metabolite of nitro group reduction in parathion, forms 4-aminophenol by hydrolysis. 4-Nitrophenol forms 4-aminophenol upon reduction. Incubation of soils with [^{14}C]parathion or 4-nitro[^{14}C]phenol resulted in each case in the formation of 4-amino[^{14}C]phenol[2,3]. Furthermore, aminophenols including 4-aminophenol are known metabolites of aniline[4].

The high-performance liquid chromatographic (HPLC) determination of 4-aminophenol has previously been reported[5,6]. Detection was performed by spectrophotometric monitoring. The fluorescence characteristics of 4-aminophenol have not yet been utilized for its detection, although 4-aminophenol has fluorescence characteristics of the same order of magnitude as phenol and alkylphenols, for which compounds HPLC combined with fluorescence detection has been reported[7].

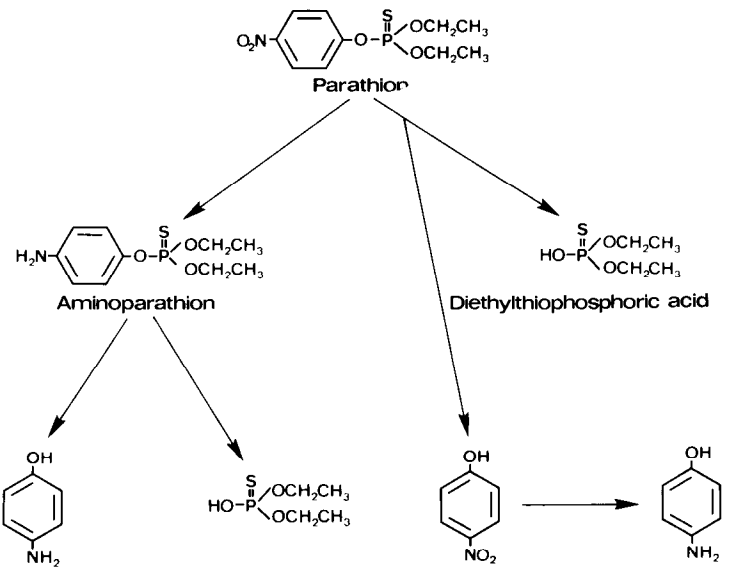

Fig. 1. Formation of 4-aminophenol due to parathion metabolism in microbial, plant and animal sources[1].

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The present paper describes the determination of 4-aminophenol in water by ion-pair HPLC and fluorescence detection. This method allows direct determination of 4-aminophenol in tap-water and surface water at low μg/l concentrations (ppb level).

EXPERIMENTAL

Chemicals

4-Aminophenol was obtained from Merck (Darmstadt, F.R.G.), 4-nitrophenol from Fluka (Buchs, Switzerland), methanol and ascorbic acid from May & Baker (Dragenham, U.K.) and $5 \cdot 10^{-3}$ *M* tetrabutylammonium phosphate buffered at pH 7.5 (PIC-A reagent) from Waters Assoc. (Milford, MA, U.S.A.). Stock solutions of 4-aminophenol were prepared in methanol–water (50:50) containing ascorbic acid (1 mg/ml).

High-performance liquid chromatography

A liquid chromatograph consisting of a Waters Model 6000 A pump, a Waters U6K injector and a Perkin-Elmer 650-40 fluorescence spectrophotometer was used. The excitation and emission wavelengths of the detector were set tot 323 and 373 nm, respectively, with the bandpass for each monochromator set to 15 nm. A Merck LiChrosorb RP-8, 5-μm column (125 × 4.6 mm I.D.) was used. It was operated at 20°C with 30 ml of PIC-A reagent in 1 l water–methanol (80:20, v/v) as the mobile phase at a flow-rate of 1 ml/min. Sample injections of 100 μl were performed.

Fluorescence spectra

Excitation and emission spectra of 4-aminophenol were obtained at a concentration of 1 μg/ml in water. The bandpass for both monochromators was set to 5 nm and a scan speed of 60 nm/min was used. The excitation spectrum was obtained with an emission wavelength of 373 nm, the emission spectrum with an excitation wavelength of 323 nm. The Perkin-Elmer 650-40 spectrofluorimeter was used with a standard cuvette accessory instead of the flow cell accessory used for the HPLC analysis.

Sample preparation

Water samples were analyzed directly by injection of 100 μl.

RESULTS AND DISCUSSION

Maximum excitation and emission wavelengths for 4-aminophenol were found to be 323 and 373 nm, respectively. Phenol has excitation and emission maxima at 276 and 298 nm, respectively. The relationship between the excitation and emission maxima for 4-aminophenol and phenol is in accord with the fact that substitution of an amino group into an aromatic system increases the excitation and emission maxima[8]. Furthermore, substitution with amino groups is known to increase fluorescence intensity[8].

The detection limit (signal/noise ratio of 3) using fluorescence detection of 4-aminophenol was found to be 300 pg. Injection of 100 μl water sample then gives a detection limit for 4-aminophenol in water corresponding to 3 μg/l (3 ppb). The

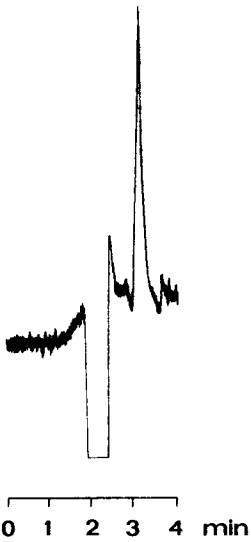

Fig. 2. High-performance liquid chromatogram of a surface water sample from a lake spiked with 4-aminophenol at a concentration of 10 μg/l (10 ppb). Column: LiChrosorb RP-8, 5 μm (125 × 4.6 mm I.D.). Mobile phase: 30 ml PIC-A reagent in 1 l water–methanol (80:20); flow-rate, 1 ml/min. Fluorescence detection: excitation, 323 nm; emission, 373 nm.

analysis of tap-water and surface water did not show any interference, giving the same detection limits in these media as in Millipore-filter water as illustrated in Fig. 2.

Regression analysis showed a linear correlation between the injected amount and the fluorescence intensity for 4-aminophenol in the range 0.3–3 ng:

$$y = -0.0360 + 3.69x$$

where x = amount (ng) injected and y = maximum peak height (cm); the correlation coefficient was 99.9%. A relative standard deviation of 0.4% (n = 6) was obtained for direct determination of 4-aminophenol at a concentration of 5 μg/l in tap-water.

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