Determination of Diaminopyrenes by High Performance Liquid Chromatography with Chemiluminescence Detection

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Dinitropyrenes (DNPs) are known mutagenic nitro polycyclic aromatic hydrocarbons (NPAHs) in the environment. Analysis of DNPs present in the environment, however, is difficult because of their low levels. It is known that DNPs are chemically or electrochemically reduced to highly fluorescent diaminopyrenes (DAPs) and that several fluorescent compounds are determined by high performance liquid chromatography (HPLC) more sensitively and selectively with chemiluminescence (CL) detection than fluorescence (FL) detection. We therefore developed a HPLC method with CL detection for the trace level analysis of DAPs and used it for determination of DNPs in environmental samples.

The degradation of DAPs due to exposure to daylight and in the presence of stainless steel disturbed this sensitive determination. Addition of ascorbic acid to the sample at a concentration of 10 mM resulted in a remarkable increase in DAP peaks. This suggests that the degradation of DNPs is ascribed to an oxidative reaction.

The HPLC system consisted of two pumps, an injector with 20 μl sample loop, analytical column, and FL and CL detectors. Other HPLC conditions were as follows: mobile phase, 10 mM imidazole-perchloric acid buffer (pH 7.6) - acetonitrile at a flow rate of 1.0 ml/min. CL reagent solution, 0.02 mM bis(2,4,6-trichlorophenyl)oxalate (TCPO) - 15 mM hydrogen peroxide in acetonitrile at a flow rate of 1.0 ml/min.

The calibration curves of DAPs using CL detection showed good linearity over 2.5 orders, their correlation coefficients being 0.9996—1.0000 and slopes 0.97—1.00, respectively. The detection limits of 1,3-, 1,6-, 1,8-DAP (DAPs) were 0.25, 0.25 and 0.35 fmol, respectively, as the signal-to-noise ratio was 3. These values were 30—60 times more sensitive than those by FL detection.

The present method was used in the analysis of DNPs in sooty emissions obtained from the exhaust of a diesel-powered car. The sample was extracted with benzene-ethanol and subjected to a Sep-Pak alumina cartridge. The obtained NPAHs-fraction was reduced to amino-PAHs by sodium hydrosulfide and analyzed with the above HPLC method. 1,3-DNP (0.10 pmol/mg), 1,6-DNP (0.17 pmol/mg), 1,8-DNP (0.24 pmol/mg) and 1-NP (19 pmol/mg) were detected from the sample.