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Short Communication

Sensitive fluorescence detection of some nitrosamines by precolumn derivatization with dansyl chloride and high-performance liquid chromatography☆

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ABSTRACT

A fluorescence detection method for the determination of some nitrosamines by precolumn derivatization with dansyl chloride and high-performance liquid chromatography was developed. The separation, derivatization and detection conditions were optimized. The sample is first denitrosated by hydrobomic acid–acetic acid to produce secondary amines, which are then subjected to reaction with dansyl chloride to form dansyl derivatives. The reaction mixtures are injected and separated on a C_{18} column with acetonitrile–water (73:27, v/v) as mobile phase with fluorimetric detection at 530 nm (excitation at 350 nm). The method shows high sensitivity and precision. The detection limits are 0.06 ng for N-nitrosodimethylamine and N-nitrosodiethylamine, 0.09 ng for N-nitrosodipropylamine and 0.16 ng for N-nitrosodibutylamine, with relative standard deviations of 2.1, 1.9, 2.1 and 1.5%, respectively. The linearity of the calibration graphs covers more than two orders of magnitude of concentration of the compounds, and the correlation coefficients are 0.9995.

INTRODUCTION

The investigation of nitrosamines is a topic of growing importance because of their carcinogenicity. High-performance liquid chromatography (HPLC) is powerful technique for the analysis of this type of compound, and several HPLC methods [1–5] for the detection and determination of N-nitroso compounds have been reported. Reversed-phase HPLC with UV detection [1] has been used for the quantitative detection of some nitrosoamines. However, this technique lacks both the sensitivity and selectivity necessary for trace analyses of environmental samples. In an attempt to solve the selectivity problem, Singer *et al.* [2] developed a specific method in which a postcolumn reaction detection system is used for HPLC. This reaction detector is useful for those compounds which can be hydrolysed in a dilute acidic solution to give the nitrite ion. This method involves the use of Griess reagent in the postcolumn reactor for the production of chromophores from the N-nitrosamines. The theoretical detection limit for this method was reported to be 0.5 nmol and it is specific for all N-nitroso compounds cleaved by dilute hydrochloric acid. However, owing to the slow reaction kinetics of some nitroso compounds, this technique requires both an air segmentation system and a high-temperature reactor.

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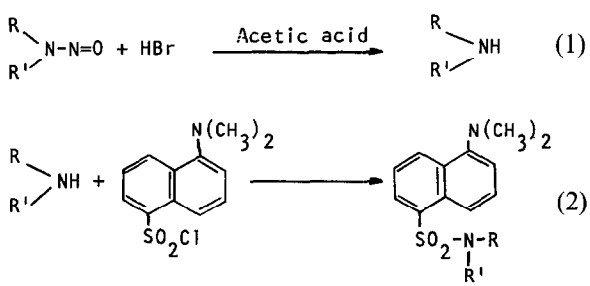
HPLC–thermal energy analysis methods [3,4] have been reported for the determination analysis of some nitrosamines. However, in order to operate continuously, this techniqe requires the use of cold traps for the removal of the eluent and the by-products of pyrolysis and the employment of microbore columns, which limits the use of the method. Lee and Field [5] reported a selective fluorescence detection method for the determination of some N-nitrosamines after postcolumn reaction. The nitrosamines eluted from the column are first hydrolysed to produce the nitrite anion, which is then oxidized with Ce^{4+} to give fluorescent Ce^{3+}. The detection limit for this method is at the ppb level. However, this method also requires a complex postcolumn reactor system with a heating bath.

We report here a sensitive precolumn derivatization method for some nitrosamines, which is based on the following reaction:



The sensitivitiy and the precision of the method are satisfactory and, as it involves only the use of conventional instrumentation and reagents, it is easy to employ.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a constant-flow pump (Shanghai Chemical Industry Training School, Shanghai, China), an RF-535 fluorescence HPLC monitor (Shimadzy, Kyoto, Japan), a Perkin-Elmer Model 56 recorder and a Rheodyne (Berkeley, CA, USA) Model 7105 injection valve. The separation was carried out on a Perkin-Elmer C_{18} column (12.5 cm × 4.6 mm I.D.).

Chemicals

N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA) and N-nitrosodibutylamine (NDBA) were prepared by conventional procedures. All other reagent were of analytical-reagent grade. High-purity water was obtained by double distillation. A mixed standard solution containing 0.1 mg of each of the four N-nitrosoamines in 1 ml of dichloromethane was first prepared, and dilution was made as required. Hydrobromic acid–acetic acid reducing reagent was prepared by adding 5 ml of of acetic anhydride to 4 ml of 47% hydrobromic acid. Dansyl chloride solution was prepared by weighing 28 mg of dansyl chlorid (E. Merck) into a 10-ml volumetric flask, diluting to the mark with acetone and mixing well.

Preparation of dansyl derivatives

A 100-μl volume of the standard solution was mixed with 5 μl of reducing reagent in a graduated test-tube with stopper. After denitrosation reaction for 10 min at 40°C, the stopper was removed in a hood and the dichloromethane solvent was evaporated. About 60 μl of 1 mol/l NaOH solution were added to adjusted the pH to about 9, followed by the addition of 0.5 ml of 0.2 mol/l $NaHCO_3$ buffer and 0.5 ml of dansyl chloride solution. After 30 min at 40°C, the derivatives were diluted with water to 2 ml for HPCL analysis. A blank was run at the same time.

Analytical conditions

The mobile phase was acetonitrile–water (73:27, v/v) at a flow-rate of 0.8 ml/min. The injection volume was 20 μl and the chart speed was 5 mm/min. The fluorescence excitation wavelength was 350 nm and the emission wavelength was 530 nm.

RESULTS AND DISCUSSION

Selection of excitation and emission wavelengths

Stopped-flow scanning was applied to the peak of each of the derivatives to obtain both the emission and excitation spectra. The results obtained showed that the excitation and emission maxima of the NDEA, NDPA and NDBA were at 350 and 530 nm, respectively, but the fluorescence emission maximum of the NDMA derivative was at 540 nm. This is perhaps caused by the hyperconjugation effect in the structure of the NDMA derivative. Con-

sidering the sensitivity as a whole, an emission wavelength of 530 nm and an excitation wavelength of 350 nm were selected for the detection.

Separation of derivatives

Acetonitrile–water mixtures were tested as the mobile phase. When the content of acetonitrile in the mobile phase was increased, the capacity factors (k') of all four derivatives decreased. On the basis of both resolution and separation time, the optimum acetonitrile content was chosen as 73%. A typical chromatogram is shown in Fig. 1, indicating a good separation.

Denitrosation reaction time

The peak heights of the derivatives were measured for denitrosation reaction times from 5 to 40 min at 40°C, other conditions being identical. The peak heights for the derivatives remained almost constant. A denitrosation reaction time of 10 min was adopted.

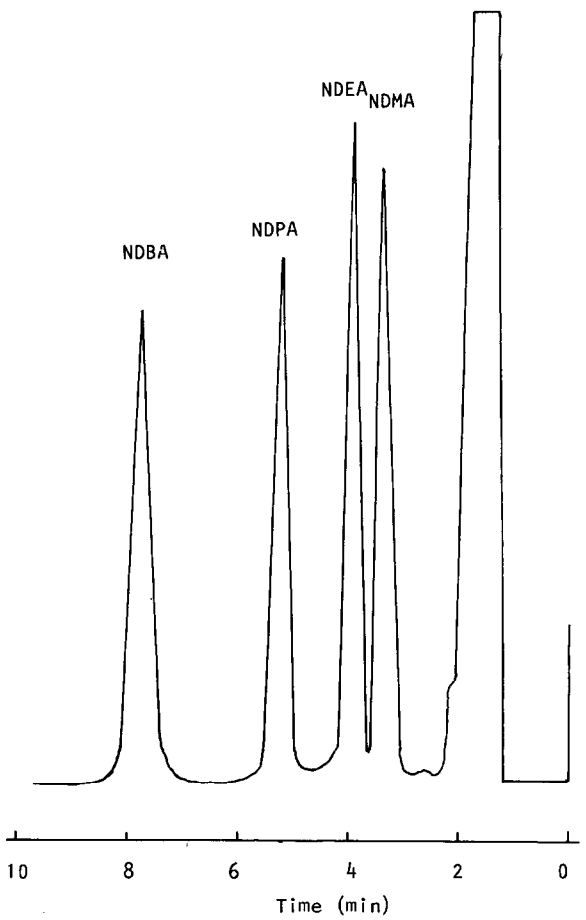


Fig. 1. Chromatogram of derivatives of nitrosamines.

Effect of amount of reducing reagent on signal

With other conditions were fixed, the peak heights of the derivatives were measured with various amounts of the reducing agent. The results showed that with between 1 and 15 μl of the reducing agent, the peak height of the derivatives remained almost unchanged, but above 15 μl the peak height decreased and fluctuated. This effect can be explained as follows. As the amount of reducing agent added is increased, adjustment of the pH to 8.5, which is necessary for the subsequent dansylation reaction, will result in the electrolytes in the reaction media being increased considerably. When the amount of reducing agent added is more than 15 μl, the amount of electrolytes formed will be large sufficient to interfere with the dansylation reaction and therefore make the signal irregular. Owing to the high concentration of the reducing agent, even 1 μl can suffice for the denitrosation reaction. The addition of 5 μl of the reducing agent was selected for subsequent experiments.

Effect of dansylation time on signal

The results showed that the peak height of the derivatives increased rapidly up to 20 min, and then remained constant as the reaction time increased. A derivatization time of 30 min was chosen for subsequent work.

Effect of amount of dansyl chloride on signal

The effect of the amount of dansyl chloride in the range 0–600 μl on the peak heights was investigated. The results showed that the peak height of the derivatives increased markedly as the amount of dansyl chloride was increased from 0 to 200 μl, but above 200 μl it remained almost unchanged. As a result, 500 μl of the dansyl chloride were selected for subsequent experiments.

Calibration and detection limits

A regression analysis was made for all four calibrations, and the relative standard deviations were obtained from seven replicate measurements. The results showed that the correlation coefficients for the

four nitrosamines exceed 0.9995 in the range 1–100 ng (linearity outside this range was not investigated); the detection limits (signal-to-noise ratio = 2) with this technique were between 0.06 and 0.16 ng, depending on the compound; the relative standard deviations for NDMA, NDEA, NDPA and NDBA (peak height) were 2.1, 1.9, 2.1 and 1.5%, respectively.

The sensitivity of this method is good, and the detection limit is lower than those reported for other methods [2,5]. The proposed method also does not require the complex postcolumn reaction apparatus needed for previous methods and can be readily used with conventional instrumentation and reagents. Such advantages suggest wide applicability, and the method provide a good approach for the detection of trace nitrosamines in environmental samples. Pretreatment of the samples would, of course, be required before the precolumn derivatization.

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