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Short communication

Sensitive determination of alkoxyethanols by pre-column derivatization with 1-anthroylnitrile and reversed-phase high-performance liquid chromatography

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Abstract

A new method for simultaneous determination of alkoxyethanols (2-methoxyethanol, 2-ethoxyethanol, 2-isopropoxyethanol, and 2-butoxyethanol) by high-performance liquid chromatography (HPLC) with fluorescence detection has been developed. The alkoxyethanols and an internal standard (2-phenoxyethanol) were derivatized by treatment with 1-anthroylnitrile to give the anthroyl esters. The esterification was completed in 30 min in the presence of quinuclidine as base catalyst at room temperature. After stopping the reaction, an aliquot of the final solution was injected into the HPLC. The resulting anthroyl esters of the alkoxyethanols and the internal standard were separated on a C_{18} reversed-phase column with acetonitrile–water–acetic acid (65:35:0.1, v/v) as the mobile phase and detected fluorimetrically at excitation and emission wavelengths of 360 nm and 460 nm, respectively. The detection limits of the derivatives as alkoxyethanols at a signal-to-noise ratio of 3 were in the range of 1–3 pg per injection. The minimal amounts of alkoxyethanols derivatized in the reaction mixture for derivatization to determine the limits of detection were approximately 0.5 ng. This HPLC method was applied to the determination of some of alkoxyethanols in the air of the workplace where the thinner containing alkoxyethanols was used for painting.

Keywords: Derivatization, LC; Alkoxyethanols; Alcohols; Anthroylnitrile

1. Introduction

It is well known that alkoxyethanols, in particular 2-methoxyethanol (ME), 2-ethoxyethanol (EE), and 2-butoxyethanol (BE), can cause adverse reproductive, developmental, and hematological effects through inhalation, dermal absorption, and ingestion [1–5]. Alkoxyethanols are widely used in industry as solvents in the manufacture of lacquers, varnishes, resins, and printing inks owing to their excellent hydrophilic and lipophilic properties.

Environmental monitoring of the alkoxyethanols in the air of the workplace where the alkoxyethanols are used is required to obtain information on working environments. Gas chromatography (GC) is the most widely used method for quantification of the alkoxyethanols in the air of the workplace. The GC methods [6–8] for the alkoxyethanols with flame ionization detection (FID), however, are not suffi-

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ciently sensitive for short-term sampling, for example 10 or 15 min sampling time, because the reliable measurement limit of detection of ME is 4 ng per injection [8]. Therefore, the airborne alkoxyethanols at a concentration of p.p.b. level can not be detected with sufficient sensitivity and precision by means of the GC–FID following short-term sampling.

Several pre-column derivatization methods [9–15] have been reported for the derivatization of alcohols using high-performance liquid chromatography (HPLC) with ultraviolet detection or fluorescence detection. For most of these methods, the derivatization procedure involves heating in an anhydrous solvent. Moreover, most of the reagents, both in their native form and as their alcoholic derivatives, are unstable. Goto et al. [16] have developed a new type of fluorescent derivatization reagent for hydroxysteroids, that is, 1-anthroylnitrile (1-AN) generated anthroyl esters. The reagent has been reported to be useful and sensitive for the determination of alcohols under mild conditions. However, there has been no study that tried to apply the reagents for alkoxyethanols.

Here, we report the use of 1-AN as a very sensitive reagent for derivatization of alkoxyethanols. The dependence of the derivatization procedure on reaction conditions, the separation conditions for the derivatized alkoxyethanols, and the quantitative analytical characteristics of the method are described. Using this method, we determined EE and BE in the air of a workplace.

2. Experimental

2.1. Materials and reagents

ME (purity >98%), EE (purity >98%), 2-isopropoxyethanol (IPE, purity >99%), BE (purity >99%), 2-phenoxyethanol (PhE, purity >99%), 1-AN, anhydrous acetonitrile and dichloromethane were purchased from Wako Pure Chemical Industries (Osaka, Japan) and they were of analytical-reagent grade. Acetonitrile used for the HPLC mobile phase was of liquid-chromatography grade. PhE was used as an internal standard. Quinuclidine was obtained from Aldrich (Milwaukee, WI, USA). Molecular sieves 0.3 nm beads (catalog no. 105704) and 0.4 nm beads (catalog no. 105708) were obtained from Merck (Darmstadt, Germany) and were added to the organic solvents to remove moisture before use.

The stock solutions of each alkoxyethanol were prepared in acetonitrile at a concentration of about 10 mg/ml. The mixed standard solution containing the alkoxyethanols at concentrations of 2.40 μg/ml for ME, 2.31 μg/ml for EE, 2.26 μg/ml for IPE, and 2.24 μg/ml for BE was prepared by dilution with a solution of 10% (v/v) acetonitrile in dichloromethane of the stock solutions. The internal standard solution (2.22 μg/ml) was prepared by dilution with acetonitrile of the stock solution (11.1 mg/ml). Both standard solutions were used for making the derivatization procedure most suitable. The mixed standard solution and the internal standard solution were diluted further for calibration and measuring limit of detection with 10% (v/v) acetonitrile in dichloromethane. Reagent solutions of 1-AN and quinuclidine were prepared by dissolving them in anhydrous acetonitrile and adding 3 g of molecular sieves 0.3 nm for 100 ml of each solution to remove moisture, to obtain a final concentration of 0.1% (w/v) and 0.5% (w/v), respectively. These reagent solutions were stable for at least 3 months in a refrigerator at 4 °C.

2.2. Derivatization procedure

The reaction of derivatization proceeded as shown in Fig. 1. One hundred microliters volume of acetonitrile, 100 μl of 0.5% (w/v) quinuclidine solution, 100 μl of the working internal standard solution, and 100 μl of the working mixed standard solution containing the alkoxyethanols or an environmental sample were placed in a test tube. Then 100 μl of 0.1% (w/v) 1-AN solution was added to the test tube to start the reaction of derivatization. The reaction mixture was allowed to stand for 30 min at room temperature for derivatization of the alkoxyethanols and the internal standard. The reaction was terminated by the addition of 3 ml of acetonitrile and the test tube containing the final solution covered with aluminum foil was kept at room temperature, and then 10 μl of the final solution was injected into HPLC within 2 h.

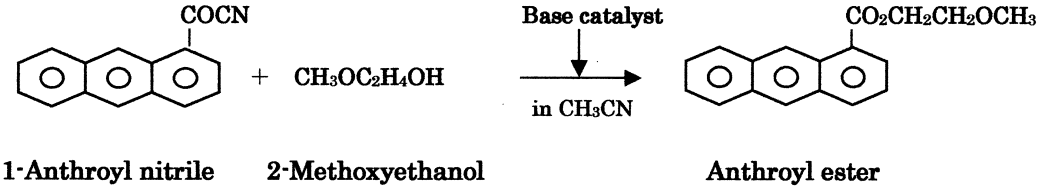

Fig. 1. The derivatization reaction of 2-methoxyethanol with 1-anthroylnitrile.

2.3. Separation of anthroyl derivatives by HPLC

The HPLC system consisted of the L-7100 pump, the L-5030 column oven, the L-7480 fluorescent detector, the L-7500 data processor (Hitachi, Tokyo, Japan) and the Rheodyne 7725i manual injector with a 20 μl loop (Rainin, Emeryville, CA, USA).

The separation of the anthroyl derivatives of the alkoxyethanols and the internal standard was performed on a C_{18} reversed-phase column (Wakosil-II $5C_{18}$HG, 150 mm×4.0 mm I.D., 5 μm particle size; Wako). The mobile phase was acetonitrile–water–acetic acid (65:35:0.1, v/v). The temperature of the column oven and the flow-rate were maintained at 35 °C and 1.0 ml/min, respectively. The column eluent was monitored with the fluorescent detector at excitation wavelength of 360 nm and emission wavelength of 470 nm. The quantification of the alkoxyethanols was done using the ratio of the peak area of the derivatives of the alkoxyethanols to the peak area of the internal standard.

2.4. Generation of EE standard gas

EE standard gas at a concentration of 4 p.p.m., which is close to the threshold limit value (5 p.p.m.) adopted by the American Conference of Governmental Industrial Hygienists (ACGIH) [17] and by the Japan Society for Occupational Health [18], was generated by using a Permeater PD-1B (Gastec, Kanagawa, Japan). The EE standard gas was introduced into a 25-l Tedlar bag and the concentration of the EE standard gas in the bag was determined periodically by means of a gas chromatograph (Hitachi, Tokyo, Japan) equipped with FID to check on the concentration of EE standard gas.

2.5. Measurement of desorption efficiency

To ensure the effective performance of coconut shell charcoal tubes (100/50 mg, Sibata Scientific Technology, Tokyo, Japan) for working environmental monitoring, a suitable desorbing solvent was selected for the alkoxyethanols by the phase equilibrium method. The mixed standard solution (1.12–1.20 μg/ml) in desorbing solvents, 5–30% (v/v) acetonitrile in dichloromethane, was prepared. One milliliter of the mixed standard solution was distributed into seven 2-ml vials. To five vials of the seven was added 100 mg of charcoal, and then all the vials were then sealed with PTFE-coated aluminum caps. After 1 h standing at room temperature with occasional vibration, the sample solutions were analyzed by the present HPLC method. The ratio of the concentration equilibrated with charcoal and the corresponding concentration without charcoal was taken as the desorption efficiency.

2.6. Preparation of environmental samples

Environmental samples in the workplace where the thinner containing EE, BE, 2-butanol, methyl isobutyl ketone, and ethyl acetate was used for painting were collected by drawing air through the charcoal tubes with personal sampling pumps at air flow-rate of 0.2 l/min for 10 min. All the charcoal tubes were stored at 4 °C until determination. The collected alkoxyethanols on the charcoals were desorbed with 1 ml of 10% (v/v) acetonitrile in dichloromethane by standing for 30 min at room temperature with occasional vibration. One hundred microliters of the resulting sample was added to the reaction mixture for derivatization of the alkoxy-

ethanols, and then the anthroyl derivatives yielded were measured by the present HPLC method.

3. Results and discussion

3.1. Conditions of derivatization

Triethylamine and quinuclidine have been used as a base catalyst for esterification of hydroxyl group with 1-AN [14–16]. Quinuclidine was selected as the catalyst in this study because quinuclidine solution gave the best result as assessed by the detector response among the two base catalysts and mixtures of the two tested. Then the optimal conditions, concentration of quinuclidine solution, reaction temperature, and reaction time for the derivatization of the alkoxyethanols with 1-AN were examined using the mixed standard solution (2.24–2.40 μg/ml) and the internal standard solution (2.22 μg/ml).

The effects of the concentrations of quinuclidine solution on fluorescence response of the anthroyl derivatives were tested. The fluorescence responses of the alkoxyethanols and the internal standard were the maximum over the concentration range from 0.5% (v/v) to 1% (v/v), and decreased at the concentration of 2.5% (v/v) compared to those at 0.5% (v/v)–1.0% (v/v). Therefore, 0.5% (v/v) quinuclidine solution was used as a base catalyst.

A fixed quinuclidine concentration of 0.5% (v/v) was used as a catalyst, and the effects of reaction temperature on the fluorescence response were examined. Consequently, the fluorescence responses of the anthroyl esters at room temperature were greater than those at 40 °C and 60 °C.

The effects of reaction time on the fluorescence response were examined under the conditions of the 0.5% (v/v) quinuclidine solution and at room temperature. The fluorescence responses reached maximum over the range of reaction time from 30 min to 90 min and were constant. From these results, we decided that the optimal conditions for derivatization of the alkoxyethanols for concentration of catalyst, reaction temperature, and reaction time were 0.5% (v/v) quinuclidine solution, room temperature, and 30 min, respectively.

3.2. HPLC separation of anthroyl derivatives

For the simultaneous separation of the derivatives of the alkoxyethanols and the internal standard, optimal HPLC conditions were examined using a C_{18} reversed-phase column. The 65% (v/v) acetonitrile aqueous solution containing 0.1% (v/v) acetic acid at a flow-rate of 1 ml/min, when column temperature was kept at 35 °C, gave the best separation with the shortest retention times. The derivatization reagent themselves showed only feeble fluorescence at 460 nm, but an excess of the derivatization reagent and its hydrolysis product eluted together with retention time of about 3 min, as shown in Fig. 2A. The retention times of the anthroyl derivatives of ME, EE, IPE, BE, and PhE (internal standard) were 5.0, 6.7, 8.9, 14.3, and 12.1 min, respectively. Under these HPLC conditions, the anthroyl derivatives of the alkoxyethanols and the internal standard were completely separated within 20 min, and no interfering peaks were detected at the retention times of the derivatives, as shown in Fig. 2B.

In order to identify the liquid chromatographic peaks (Nos. 1–5) shown in Fig. 2B, the eluate of the LC peaks were collected. After extraction and concentration of the anthroyl derivatives from these fractions, negative-ion fast atom bombardment (FAB) mass spectra (glycerol as a matrix) were obtained with a mass spectrometer. Peak 2 was identified as a derivative of EE because the quasi-molecular ion, $[M–H]^-$ at m/z 293, was clearly detected. The other peaks were identified as the derivatives of the alkoxyethanols in the same way.

3.3. Calibration curve, limits of detection, and precision

Linear relationships were observed between the ratios of the peak area of the derivatives of the alkoxyethanols and those of the derivative of the internal standard, and the amounts of the alkoxyethanols at the concentration up to 2.40 μg/ml. These results indicate that PhE can be used as a suitable internal standard. The calibration curves of the alkoxyethanols for environmental samples were linear and passed through the origin with a correlation coefficient of 0.998 or better in the concen-

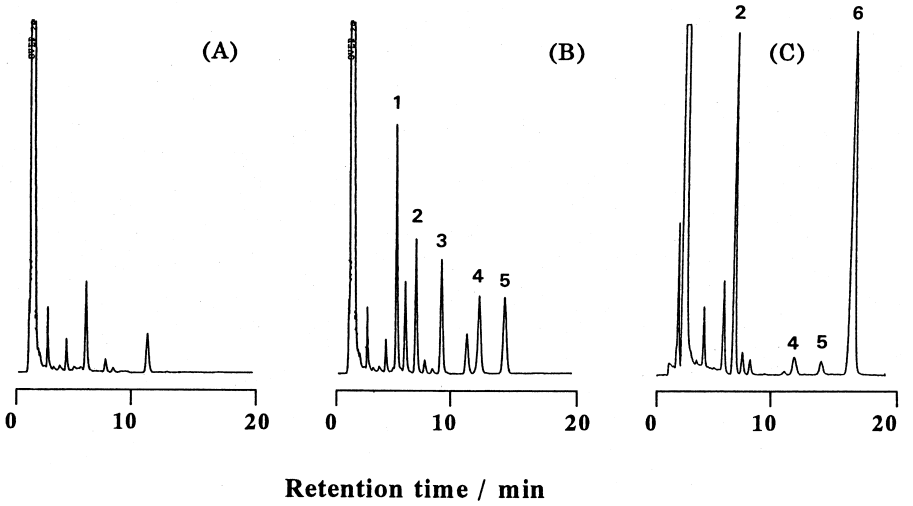

Fig. 2. High-performance liquid chromatograms obtained from (A) a reagent blank, (B) a mixed standard solution of alkoxyethanols, and (C) an environmental sample. Peaks: 1; ME ester, 2; EE ester, 3; IPE ester, 4; PhE (I.S.) ester, 5; BE ester, and 6; 2-butanol ester. Amounts of peak areas of the derivatives in the chromatogram (B) were 320–342 pg as the alkoxyethanols.

tration range from 0 to 240 ng/ml for the alkoxyethanols.

Three working standard solutions for measuring limits of detection of the alkoxyethanols at concentrations of 4.5–4.8 ng/ml, 9.0–9.6 ng/ml, and 22–24 ng/ml were prepared, and limits of detection were determined by measuring the peak heights of the anthroyl esters and the height of the baseline noise. The limits of detection of the anthroyl derivatives observed were in the range of 1–3 pg as the alkoxyethanols and PhE at a signal-to-noise ratio of 3, when a 20 μl volume of the final solution was injected into the HPLC. The limits of detection showed the minimal amounts of the alkoxyethanols derivatized in the reaction mixture for derivatization to determine the limits of detection were approximately 0.5 ng. The limits of detection of the alkoxyethanols of the present HPLC method are at least 100 times lower than that of the GC–FID method [8]. Therefore, the present HPLC method enables one to carry out short-term sampling at a concentration of p.p.b. level of the airborne alkoxyethanols.

The within-run reproducibility of the analytical procedure was examined by determining the mixed standard solution (112–120 ng/ml) in six replicates. The within-run reproducibility of the analytical procedure was good because the RSD for the peak area ratio of the derivatives of the alkoxyethanol to the derivative of the internal standard obtained were in the range of 1.2 to 1.8%.

The between-run reproducibility was also determined by the analysis of the mixed standard solution on five separate days. The RSDs for the peak area ratio of the derivatives of the alkoxyethanols to the derivative of the internal standard obtained were 1.1% for ME, 1.4% for EE, 1.4% for IPE, and 1.0% for BE, respectively.

3.4. Stability of final solutions

To study the stability of the anthroyl derivatives in final solution, the mixed standard solution (112–120 ng/ml) was derivatized using the analytical procedure. This repeatability test was performed in 13 measurements over 6 h. Between sample injections, the final solution was kept at room temperature at 25 °C. The RSDs for the peak area of the derivatives of both alkoxyethanols and internal standard were less than 2%. No interfering peaks for the determination of the derivatives were detected on the chromatograms within 6 h.

3.5. Desorption efficiency

The mixture of 5% (v/v) methanol in dichloromethane has been used as an effective desorbing solvent of alkoxyethanols from charcoal [6–8]. However, methanol can not be used in the derivatization procedures of the present method because methanol reacts with 1-AN in the presence of quinuclidine and the corresponding derivative is formed. Acetonitrile was then tested for a constituent of desorbing solvent instead of methanol. The desorption efficiencies of ME from the charcoal with 1 ml of the various mixtures of acetonitrile and dichloromethane are shown in Fig. 3. The 10% (v/v) acetonitrile in dichloromethane gave the best desorption efficiency, with 95.9±1.93% in five determinations. The desorption efficiencies of three other alkoxyethanols with 1 ml of 10% (v/v) acetonitrile in dichloromethane in five determinations were 94.6±1.33% for EE, 96.5±2.01% for IPE, and 95.8±0.76% for BE, respectively. Therefore, the mixture of 10% (v/v) acetonitrile in dichloromethane was used as a suitable desorbing solvent.

3.6. Reliability of the present HPLC method

The EE standard gas in the Tedlar bag at a concentration of 4 p.p.m. was collected by drawing the standard gas through the charcoal tubes at air flow-rate of 0.2 l/min for 10 min. Then, the concentration of EE in the standard gas was measured by means of the present HPLC method and the GC–FID method [6], to evaluate the reliability of the present HPLC method. The concentrations of EE in the standard gas obtained by both HPLC and GC–FID methods were 4.0±0.1 p.p.m. (n=5) and 4.0±0.2 p.p.m. (n=5), respectively. The results obtained by both methods were in good agreement.

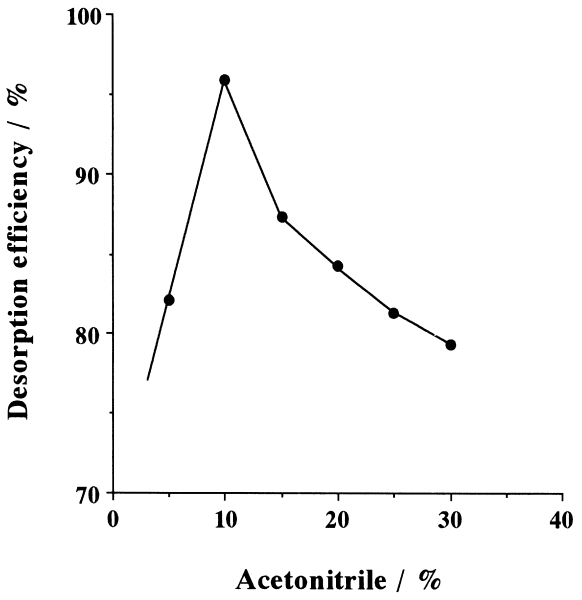

Fig. 3. Desorption efficiencies of 2-methoxyethanol from charcoal with various mixtures of acetonitrile and dichloromethane.

3.7. Application for environmental monitoring

At a workplace where thinner containing EE, BE, 2-butanol, methyl isobutyl ketone, and ethyl acetate was used, environmental monitoring of airborne EE and BE was carried out using the charcoal tubes. Stationary samplings were performed with a flow-rate of 0.2 l/min, and the sampling time was 10 min. Organic vapors absorbed were desorbed from the charcoals with 1 ml of 10% (v/v) acetonitrile in dichloromethane. These desorbing solvents were used as a sample solution for the derivatization with 1-AN. A typical liquid chromatogram obtained from the environmental sample is shown in Fig. 2C. In this environmental sample, the derivatives of EE, BE, and 2-butanol were detected and the concentrations of EE and BE in air were 150 p.p.b. and 8 p.p.b., respectively. Thus, it was found that the alkoxyethanols at the concentration of p.p.b. level in the air of a workplace could be measured with sufficient sensitivity and precision by the present HPLC method.

Since the alkoxyethanols are one of a class of chemicals exhibiting the polar properties of alcohols and the non-polar properties of ethers, some alcohols, for example methanol, 1-propanol, and 1-butanol, commonly coexist with some alkoxyethanols in raw materials. These alcohols were then examined to determine whether they react with 1-AN in the presence of the catalyst. Although they reacted with 1-AN and the corresponding derivatives were formed, peaks of derivatives of the alcohols except for 1-propanol were separate from those of the derivatives of the alkoxyethanols and the internal standard (PhE) under the HPLC conditions.

The retention time of the derivative of 1-propanol was close to that of the internal standard. However,

the present HPLC method is capable of determining the derivatives of the alkoxyethanols by selection of adequate internal standard instead of PhE, even if the alcohols coexist together in the air of a workplace.

4. Conclusions

A sensitive HPLC method for the determination of the alkoxyethanols was developed. Compared with gas chromatographic methods of alkoxyethanols and other currently available methods for the determination of alcohols, the present HPLC method has the following advantages. The derivatization reagent is commercially available. The HPLC method is simple, sensitive, reproducible and utilizes simple derivatization conditions, yielding a complete reaction within 30 min at room temperature. The liquid chromatographic conditions are simple and do not necessitate the use of complex buffered solution.

Finally, the short-term sampling using a charcoal tube and the present HPLC method can be applied for the determination of airborne alkoxyethanols at the concentration of p.p.b. level. In fact, preliminary results indicate the suitability of the present HPLC method for application to environmental samples.

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