II.1.8 Alkyl nitrites

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Introduction

Alkyl nitrites are highly volatile organic solvents of aliphatic alcohol esters of nitrites [1]. Amyl nitrite[a], butyl nitrite and isobutyl nitrite are the representative alkyl nitrites; their boiling points are 98, 78 and 67 °C, respectively. Amyl nitrite is being widely used as a detoxicant for cyanide poisoning, because alkyl nitrites oxidize hemoglobin in erythrocytes to yield methemogloblin, which is bound with cyanide to inactivate it [1]. Alkyl nitrites also show a coronary artery-dilating effect, and had been, therefore, used for the treatment of angina pectoris many years ago [2]; the pharmacological effect of the dilation of the coronary arteries was found due to the action of nitrogen monoxide produced by decomposition of alkyl nitrites [3]. They are being mainly used as materials for manufacturing drugs or as reagents for synthesis in industries; they are also used as aromatics. Because of their pharmacological effect, alkyl nitrites are being abused as uncontrolled inhalant drugs and causing a social problem [4]. Although there are many reports on toxic and fatal cases due to alkyl nitrites [5], reports on their fatal doses are few; it is estimated that oral ingestion of 10–15 mL of each alkyl nitrite causes serious methemoglobinemia [6]. The LD_{50} value for an alkyl nitrite is reported to be 205 mg/kg. There are not many cases of analysis of alkyl nitrites in the field of forensic toxicology. In this chapter, the methods for analysis of the compounds by headspace (HS)-gas chromatography (GC) and liquid-liquid extraction-GC are presented.

Determination of isobutyl nitrite in aqueous solution by headspace-GC

Reagents and their preparation

- A 115-μL volume (100 mg) of isobutyl nitrite (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan and other manufacturers) is dissolved in acetone to prepare 10 mL stock solution (10 mg/mL, preservable for a week in a refrigerator)[b]. The stock solution is diluted 2,000-fold with acetone to prepare standard solution (5 μg/mL).
- A 124-μL volume (100 mg) of isobutyl alcohol (obtainable from many manufacturers) is dissolved in acetone to prepare 10 mL stock solution (10 mg/mL, preservable in an airtight container at room temperature). The stock solution is diluted 2,000-fold with acetone to prepare standard solution (5 μg/mL).

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GC conditions

GC column: a polar fused silica capillary column (HP-Wax, 30 m × 0.25 mm i. d., film thickness 0.25 μm, Agilent Technologies, Palo Alto, CA, USA).

GC conditions[c]: an HP 6890 Series gas chromatograph (Agilent Technologies); injection mode: split with its ratio 30; injection temperature: 200 °C; detector: FID; detector temperature: 220 °C; carrier gas: He; its flow rate: 0.67 mL/min; column (oven) temperature: 40 °C (3 min) → 15 °C/min → 115 °C.

MS conditions: transfer line temperature: 280 °C; ion source temperature: 200 °C; ionization mode: EI; electron energy: 70 eV; ionization current: 60 μA.

Procedure

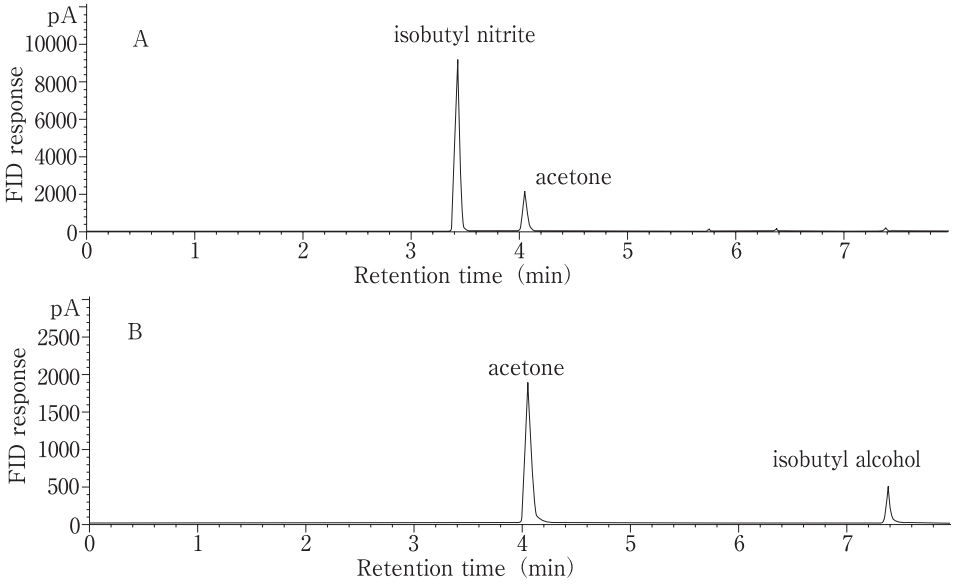
i. A 0.25-mL volume of a specimen[d], 0.5 mL of 1 M phosphate buffer solution (pH 7), 0.2 mL distilled water and 0.05 mL acetone are placed in a glass vial with a septum screw cap (8 mL volume, external diameter 17 mm, height 6 cm, GL Sciences, Tokyo, Japan) and airtightly stoppered with a cap with a Tuf-Bond™ disc (PTFE/silicone septum).
ii. The vial is incubated at 30 °C for 10 min on a Type-D aluminum block heater (Reacti-Therm ™, Pierce, Rockford, IL, USA) to gain an equilibrium.
iii. A 0.5-mL volume of the headspace vapor in the vial is drawn into a glass tuberculin syringe with a 25 G × 1″ needle (0.50 × 25 mm, Terumo, Tokyo, Japan and other manufacturers) and rapidly injected into GC.
iv. Quantitation: a 0.25 mL of the same matrix[e] (without an analyte), 0.5 mL of the phosphate buffer, 0.05 mL of isobutyl nitrite or isobutyl alcohol standard solution at various concentrations and 0.2 mL distilled water are placed in the vial and mixed. The following procedure is exactly the same as described above. The 5–6 vials containing different concentrations of the analyte are prepared to construct each external calibration curve, consisting of the concentration of an alkyl nitrite on the horizontal axis and peak area on the vertical axis. The concentration of the analyte in a specimen is calculated using the calibration curve.

Assessment and some comments on the method

Figure 8.1 shows gas chromatograms of isobutyl nitrite (injected amount 5 μg) and isobutyl alcohol (injected amount 10 μg); they appeared at 3.4 and 7.4 min of retention times, respectively. The solvent acetone appeared at about 4 min. The detection limits of isobutyl nitrite and isobutyl alcohol in liquid specimens were 62 ng and 1.9 μg/mL, respectively [7]. *Figure 8.2* shows EI mass spectra of isobutyl nitrite (A) and isobutyl alcohol (B).

Alkyl nitrites are easily hydrolyzed in aqueous solutions to yield each alkyl alcohol and inorganic nitrite; the hydrolytic reaction is even more rapid in blood [8,9]. The hydrolysis proceeds not only during storage of specimens, but also during the headspace analysis. As shown in *Fig. 8.1A*, the decomposition product isobutyl alcohol is detected even in headspace GC analysis of the standard isobutyl nitrite solution in acetone. Under acidic conditions, the esterification of inorganic nitrite, the opposite reaction, also takes place to reach an equilibri-

Figure 8.1



Gas chromatograms for isobutyl nitrite (A) and isobutyl alcohol (B).

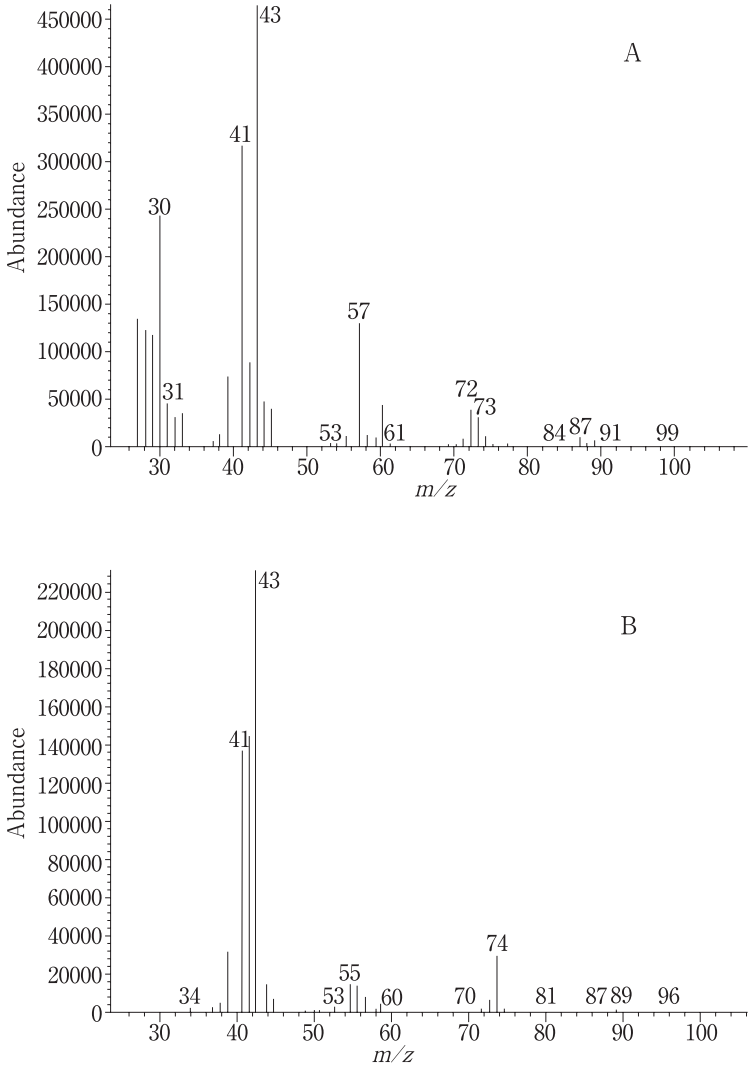
um between the hydrolysis and esterification [7]. Under the present HS conditions, the decomposition of alkyl nitrites by hydrolysis is minimized, and the esterification reaction is completely suppressed; it seems to be the best conditions for the headspace analysis of alkyl nitrites. Under these conditions, the peak of isobutyl alcohol, the decomposition product, becomes relatively low.

Determination of isobutyl nitrite in blood by GC with liquid-liquid extraction

Reagents and their preparation

- A 11.5-μL volume (10 mg) of isobutyl nitrite is dissolved in 5 mL pentane in a glass vial with a Teflon-septum screw cap to serve as a stock solution (2 mg/mL), and kept airtightly in a refrigerator. A 5-μL volume of the above solution is placed using a microsyringe in a glass vial containing 1 mL of dimethyl sulfoxide (DMSO), capped airtightly and mixed well to prepare the standard isobutyl nitrite solution (10 μg/mL).
- A 10-μL volume (10 mg) of n-propyl nitrate (Aldrich, Milwaukee, WI, USA) is dissolved in 5 mL pentane in a glass vial with a Teflon-septum screw cap to serve as a stock solution (2 mg/mL) to be stored airtightly in a refrigerator. A 5-μL volume of the above solution is dissolved in 1.0 mL DMSO in another vial; then 0.05 mL of the solution is dissolved in 4.95 mL pentane to give 100 ng/mL solution (IS-containing pentane for extraction).

Figure 8.2



EI mass spectra of isobutyl nitrite (A) and isobutyl alcohol (B).

GC conditions

GC column: a non-polar fused silica capillary column (DB-1, 30 m × 0.32 mm i. d., film thickness 1 μm, J & W Scientific, Folsom, CA, USA).

GC conditions; instrument: an HP 6890 Series gas chromatograph (Agilent Technologies); injection: split mode with its ratio of 30; injection temperature: 45 °C; detector: ECD; detector temperature: 195 °C; carrier gas: N_2; its flow rate: 1 mL/min; column (oven) temperature: 30 °C (9.5 min) → 60 °C/min → 45 °C (8.5 min).

Procedure

i. A blood specimen is directly sampled through a heparinized cannula into a 0.5-mL volume glass vial, which has been cooled with ice, capped airtightly and stored in a refrigerator.
ii. A 0.4-mL volume of the blood specimen is rapidly mixed with 0.4 mL of the IS-containing pentane solution in a glass vial with a Teflon septum screw cap, which has been cooled with ice, and vortex-mixed for 5 s.
iii. A 3-μL aliquot of the upper organic phase is rapidly injected into GC.
iv. Quantitation: the internal calibration method is used; a volume (0.5–20 μL) of the cooled standard solution of isobutyl nitrite (10 μg/mL) is added to the mixture of 0.4 mL of blank blood and 0.4 mL of IS- containing pentane solution in a glass vial with a Teflon-septum screw cap, which has been cooled with ice, and vortex-mixed for 5 s; a 3-μL aliquot of the organic layer is injected into GC. At least 5 vials with different volume of the standard isobutyl nitrite solution should be prepared to construct a calibration curve with isobutyl nitrite concentration on the horizontal axis and peak area ratio of isobutyl nitrite to IS on the vertical axis. The concentration of isobutyl nitrite in a test blood specimen is calculated using the calibration curve.

Assessment and some comments on the method

The present method is based on a report of analysis developed for studying the pharmacodynamics of isobutyl nitrite [10, 11]. In this method, every care is being taken to suppress the decomposition and evaporation of isobutyl nitrite throughout the procedure (from the sampling until injection to GC, and from the standard solution to a test specimen). In principle, the sampling, extraction and GC analysis should be made at low temperature, in a gastight state and in a short time. This method is applicable to other biomedical specimens and drinks/foods in forensic chemistry. It should be pointed out that the concentration of isobutyl nitrite detected by analysis only shows one at the time point of the injection into GC and does not reflect the *in vivo* level. Under the present GC conditions, the peak of isobutyl nitrite appears at 7.6 min and that of n-propyl nitrate (IS) at 15.9 min. The recovery of isobutyl nitrite from blood is 86 % [11].

Toxic and fatal concentrations

It is impossible to obtain fatal concentrations of alkyl nitrites in blood, because they are easily hydrolyzed in it. The inorganic nitrite, a decomposition product, is responsible for their toxicity; but it is further decomposed in a short time, after reaction with hemoglobin in erythrocytes. Therefore, the measurements of inorganic nitrite in blood seem useless for assessment of toxicity of alkyl nitrites. The most useful indicator of alkyl nitrite poisoning is methemoglobin concentrations in blood; about 20 % of methemoglobin concentration is toxic, and more than 70 % fatal [12].

Notes

a) So-called "amyl nitrite" is a mixture of pentyl nitrite isomers containing isoamyl nitrite as a main component.
b) In the commercial products of an alkyl nitrite, small amounts of corresponding alkyl alcohol, the decomposition product, are contained. Since the decomposition of an alkyl nitrite proceeds even in a polar organic solvent, such as acetone, the stock solution should be stored in a refrigerator and be used within a week.
c) There is a possibility of decomposition of alkyl nitrites in the injection chamber, column and detector, because they are very unstable. To avoid such decomposition, the temperatures of the injection port and detector were lowered in some analytical cases; however, the contamination of the injection port due to low temperature is concerned. The author tested the possibility of decomposition of the compounds in the injection chamber at various temperatures; the results showed almost no decomposition up to 200 °C. Therefore, we adopted 200 °C of injection temperature.
d) As specimens, refreshing drinks are being assumed, but this method is applicable to body fluid specimens.
e) Since a fluid matrix markedly affects the equilibration of a compound in the analysis by headspace GC [13], the same or very similar matrix should be used for constructing a calibration curve.

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