# II.8.3 Sodium azide

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## Introduction

As one of azide salts, lead azide is well known, because it is used as a detonator for air bags of automobiles. Recently, sodium azide has been frequently used for suicides and homicidal attempts. In this chapter, therefore, analytical methods are described only for sodium azide.

Sodium azide[a] is being well used as an antiseptic (preservative)[b] in biochemical researches and as a material for synthesis of organic compounds. It can be also used as a pesticide (herbicide), disinfectant for soil and antiseptic for lumbers; there had been no regulation for its use in spite of its high toxicity. However, in response to a series of imitative poisoning incidents, including many with sodium azide taking place in Japan, 1998, sodium azide was designated as a poisonous substance in January 1999; the handling and management of the compound has become strictly controlled by the Poisonous and Deleterious Substances Control Law in Japan.

## Analysis by the Conway diffusion method[c]-ion chromatography

### Reagents and their preparation

- A 0.4-g amount of NaOH is dissolved in ultrapure water to prepare 100 mL solution (0.1 M).
- A 10-mL volume of concentrated sulfuric acid is carefully diluted 10-fold with ultrapure water to prepare 100 mL (10 %) solution.

### Ion chromatographic conditions

Column: an IonPac AS15 column (25 cm × 4 mm i.d., Dionex, Sunnyvale, CA, USA); guard column: an IonPac AG15 column (5 cm × 4 mm i.d., Dionex).

Mobile phase: 40 mM KOH solution at a flow rate of 1.2 mL/min.

Instrumental conditions; instrument: a DX 500 ion chromatograph with an autosuppressor[d] (ASRS 4 mm, supplied current 100 mV); detector: a CD 20 conductivity detector (all from Dionex).

### Procedure

i. A 1.0-mL volume of 0.1 M NaOH solution is placed in the central round basin of a Conway microdiffusion cell[c].

ii. A specimen, such as fruit juice or urine, is placed in the outer groove of the cell without any dilution; blood or curry with high viscosity is diluted about 2-fold with distilled water and placed in the above groove.
iii. The Conway cell is sealed airtightly with a glass plate cover smeared with Vaseline at the joint part. By sliding the glass plate, a part of the cover above the outer groove is opened; 1.0 mL of 10 % sulfuric acid is added to the specimen placed in the groove and the cell is sealed immediately.
iv. The cell is gently moved to well mix sulfuric acid with the specimen in the groove, and left at room temperature for 1 h.
v. The NaOH solution in the central round basin is passed through a membrane filter[e] (0.45 μm for ion chromatography). A 10-μL aliquot of the filtrate is injected into an ion chromatograph.

## Assessment of the method

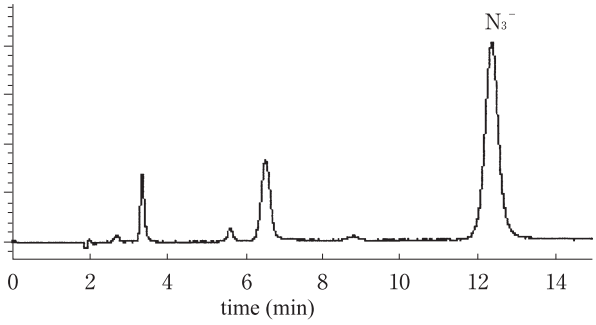
*Figure 3.1* shows an ion chromatogram obtained under the present analytical conditions. Other anion exchanger columns can be also used, if the mobile phase is optimized for each column. When the pH of a mobile phase is alkaline, the NaOH solution extract in the central basin can be directly injected into an ion chromatograph.

For foods and biological specimens, such as coffee, fruit juice, urine and blood, it is inappropriate to inject each specimen into the instrument only after dilution with distilled water or filtration; sodium azide in such a specimen is converted into gaseous hydrazoic acid, which is trapped in the NaOH solution in the Conway microdiffusion cell. This diffusion extraction method is very useful for extensive purification of azide from a crude specimen.

For a relatively clean specimen with small amounts of impurities, such as tap water or tea, it is possible to directly inject it into the instrument only after dilution plus filtration. A powder specimen should be dissolved in ultrapure water to prepare 1–10 μg/mL solution, filtered and injected into the instrument.

The detection limit oftainable by this method is about 0.1 ppm. The method is relatively simple, but the cost for the modern ion chromatographic system is more than twice of that for a usual HPLC system.

**Figure 3.1**




**Ion chromatogram for azide ion.**

## GC/MS analysis with derivatization

### Reagents and their preparation

- A 75-μL (0.13 g) or 300-μL (0.52 g) volume of pentafluorobenzyl bromide (PFBBr) is dissolved in acetone to prepare 10 mL solution (50 or 200 mM) (to be used for the below (i) procedure).
- A 30-μL volume (52 mg) of PFBBr is dissolved in ethyl acetate to prepare 10 mL solution (20 mM) (for the (ii) procedure).
- A 150-μL volume of PFBBr is dissolved in dichloromethane to prepare 10 mL solution (100 mM) (for the (iii) procedure).
- A 7.9-g amount of sodium thiosulfate is dissolved in distilled water to prepare 100 mL solution (0.5 M) (for the (i) procedure).
- A 4.2-mL volume of conc. HCl is carefully dissolved in distilled water to prepare 100 mL solution (0.5 M) (for the (i) procedure).
- A 184-mg amount of tetradecyldimethylbenzylammonium chloride (TDMBA) is dissolved in deoxygenated distilled water, which had been saturated with sodium tetraborate, to prepare 100 mL solution (5 mM) (for the (ii) procedure).
- 0.1 M Phosphate buffer solution (pH 7.7)[b] (for the (iii) procedure): 0.1 M potassium dihydrogenphosphate solution/0.1 M disodium hydrogen-phosphate solution (1:9, v/v).
- Polymer-phase-transfer catalyst (TBMPB; polymer-bound tributylmethylphosphonium bromide)[f] (for the (iii) procedure): 1.0 g of polymer-bound tributylmethylphosphonium chloride (0.90 mmol $Cl^-$/g resin, Fluka, Buchs, Switzerland) is mixed with 20 mL of 5 % (w/v) hydrobromic acid solution with stirring at room temperature for 30 min. This procedure is repeated three times by adding the new 5 % hydrobromic acid solutions. Then the polymer is washed with 20 mL distilled water 5 times, with 20 mL methanol 5 times, with 20 mL dichloromethane 3 times and with 20 mL diethyl ether 3 times, and dried under reduced pressure over 24 h.

### GC/MS conditions

Column: a slightly polar fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm).

GC/MS conditions; instrument: QP-5050 (Shimadzu Corp, Kyoto, Japan); ionization: positive EI and negative ion chemical ionization (NICI) modes; injection temperature: 250 °C; column temperature: 50 °C → 10 °C/min → 150 °C; ion source temperature: 250 °C; injection mode: split (5:1); carrier gas (flow rate): He (4.0 mL/min).

### Procedures

At the first step, qualitative analysis [1] is made by GC/MS for a specimen after its derivatization with PFBBr[g]. Quantitative analysis is then carried out using a calibration curve obtained from blank specimens spiked with various concentrations of sodium azide. There are three methods for derivatization of azide as follows.

**i. Derivatization in the acetone-water mixture in the presence of a base [2]**

i. Urine specimen: 2 mL of urine is mixed with 0.5 mL of sodium tetraborate-saturated aqueous solution and 1 mL of 50 mM PFBBr acetone solution in a 10-mL volume glass test tube with a ground-in stopper.
   Blood specimen: 0.2 mL of whole blood is mixed with 0.2 mL distilled water, 0.5 mL of sodium tetraborate-saturated aqueous solution and 0.5 mL of 200 mM PFBBr acetone solution in a 10-mL volume glass test tube with a ground-in stopper.
ii. The mixture prepared above is heated at 50 °C for 20 min. After cooling to room temperature, 1 mL of 0.5 M sodium thiosulfate aqueous solution is added to the mixture and shaken vigorously for 1 min[h].
iii. It is again heated at 50 °C for 2 min. After cooling to room temperature, 1.0 g NaCl is added to it and the final volume is adjusted to 6 mL with distilled water.
iv. After adding 1.0 mL of *n*-hexane and an appropriate internal standard (IS)[i], the mixture is shaken vigorously.
v. It is centrifuged; the organic layer is transferred to another test tube and washed with 1.0 mL of 0.5 M HCl. A 2-μL aliquot of the organic layer is injected into GC/MS.

**ii. Derivatization in a two-phase system in the presence of an ion-pairing reagent for liquid-liquid extraction [3]**

i. A 0.5-mL volume of 20 mM PFBBr ethyl acetate solution, 0.8 mL of 5 mM TDMBA solution (dissolved in sodium tetraborate-saturated aqueous solution) and an appropriate IS[i] are placed in a 10-mL volume glass test tube with a ground-in stopper.
ii. A 0.2-mL volume of urine or blood is added to the above mixture, vortex-mixed for 1 min and heated at 60 °C for 30 min.
iii. After centrifugation, a 1-μL aliquot of the organic phase is injected into GC/MS.

**iii. Derivatization in a tri-phase system using a polymer-phase-transfer catalyst [1]**

i. The pH of a specimen is adjusted to 7.5–8 with either NaOH or acetic acid aqueous solution.
ii. The specimen (0.1–1 mL), 100 μL of 0.1 M phosphate buffer solution (pH 7.7), 400 μL of 100 mM PFBBr dichloromethane solution, 30 mg of the polymer-phase-transfer catalyst TBMPB[f] and an appropriate IS[i] are placed in a 2-mL volume safe-lock tube.
iii. After shaking at 38 °C for 30 min in a water bath, it is centrifuged, if necessary, to obtain the organic phase.
iv. The organic phase is dehydrated with anhydrous sodium sulfate, and a 1-μL aliquot of it is injected into GC/MS.

## Assessment of the methods

The procedures for the present method are more complicated than that for ion chromatography. The method consists of conversion of an inorganic azide anion into pentafluorobenzyl azide ($PFBN_3$) and measurements of its mass spectrum for identification. This method is best for qualitative analysis. The derivatization can be chosen from the above (1)–(3) procedures in view of simplicity of procedure and availability of reagents. By either derivatization method, $PFBN_3$ can be obtained almost quantitatively[b, g].

In the above (1) derivatization, azide is easily derivatized in a homogenous system of water-acetone in the presence of a base. At the steps ii) and iii), excessive PFBBr is converted into water soluble pentafluorobenzyl thiosulfate ion, followed by extraction of $PFBN_3$ into an organic solvent. The influence of tailing of the PFBBr peak, which appears just before that of $PFBN_3$, can be excluded by the liquid-liquid extraction. The procedure of derivatization is relatively complicated, but it is sensitive and useful especially when the target compound is only azide.

In the above (2) and (3) derivatizations, the reactions take place in the two-phase (water-organic phases) and tri-phase (water-organic-solid phases) in the presence of an ion-pairing reagent and a polymer-phase-transfer catalyst, respectively. Both derivatization procedures enable simultaneous detection of PFB derivatives of cyanide and thiocyanate ions together with the azide ion, when these ions coexist in a specimen. Especially in the (3) derivatization, the method was optimized for simultaneous detection of the 3 anions; it is usable even for a large volume of a specimen[j] and gives a clean extract. Even when the polymer-bound tributylmethylphosphonium chloride commercially available is directly used as a phase-transfer catalyst, it gives no problems for analysis of azide and thiocyanate ions; but derivatization efficiency becomes much lower for the cyanide ion[f].

*Figure 3.2* shows positive ion mass chromatograms, and positive-ion EI and NICI mass spectra after the procedure (3) for a urine specimen (0.5 mL), into which azide ion at the concentration of 10 μg/mL and 5 μg Tetralin® (IS) had been spiked.

The detection limits in the scan mode are: 100 ng/mL (whole blood specimen, EI mode) and 10 ng/mL (urine specimen, EI mode) for the (1) procedure [2]; about 20 ng/mL (blood and urine specimen, NICI mode) for the (2) procedure [3]; 200 ng/mL (various beverage specimens, EI mode) and 10–25 ng/mL (various beverage specimens, NICI mode) for the (3) procedure [1].

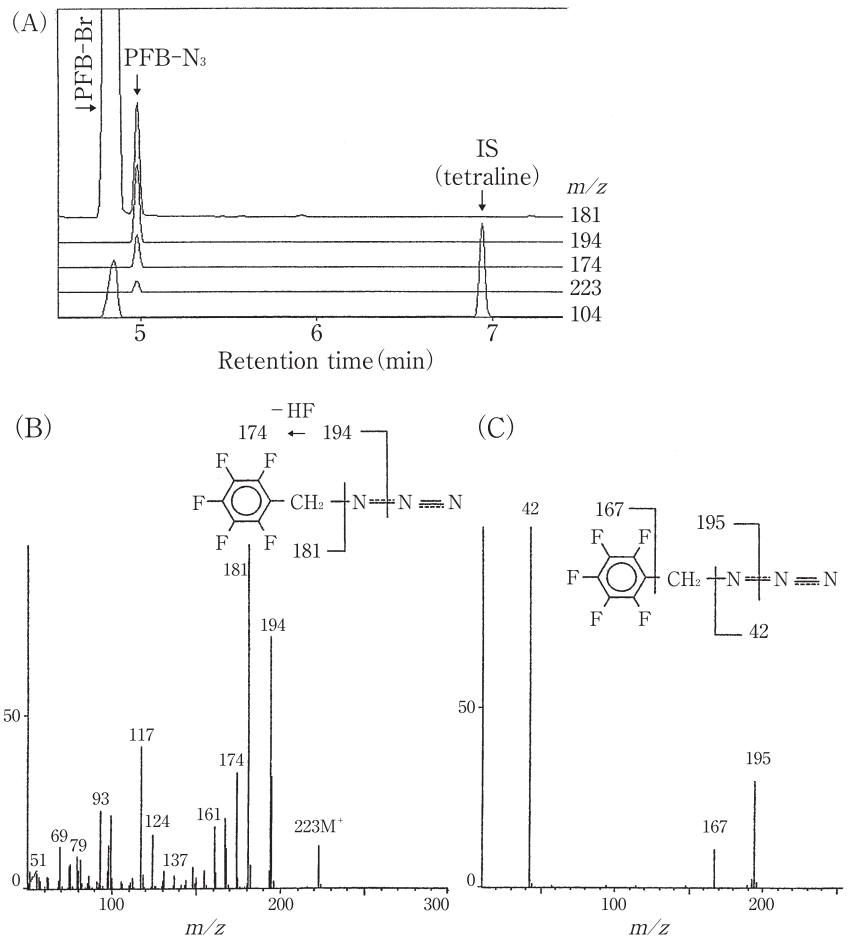
The concentration of azide, which has been added to whole blood, rapidly decrease during storage by refrigeration (4 °C), but such decrease can be prevented by adding sodium tetraborate or NaOH solution to specimens [2].

## Poisoning symptoms, and toxic and fatal concentrations

The toxicity of azide is said comparable to that of cyanide; the azide, like cyanide, is bound with the trivalent iron of cytochrome oxidase to inhibit the enzyme, causing the toxicity of azide. Its toxicity is observed after oral, percutaneous, intraperitoneal, intravenous and subcutaneous administrations. After inhalation of hydrazoic acid vapor and oral intake of azide, respiratory stimulation and tachycardia appear, followed by metabolic acidosis, hypotension, respiratory suppression, bradycardia, convulsion and finally death. Metabolism and excretion of azide in humans have not been well studied [4]. The affinity of azide ion for methemoglobin is much lower than that of cyanide ion; the detoxification of azide poisoning, therefore, cannot be achieved by the administration of sodium nitrite [5].

There was a report [6] describing a victim showing poisoning symptoms after oral intake of 5–10 mg sodium azide. Many years ago, sodium azide had been used for treatment of hypertension with a therapeutic dose of 0.65–1.3 mg. Therefore, the minimum oral toxic dose of sodium azide seems to be several mg. Until this time, a considerable number of cases of sodium azide poisoning were reported [7,8]; there was a survived case, in which as much as 150 mg of sodium azide was ingested [9]. There are reports describing fatalities with doses of 0.7–0.8 g [10] and 1.2–2.0 g [11].

**Figure 3.2**




**Analysis by GC/MS for azide ion after PFB derivatization. (A): mass chromatograms in the positive EI mode; (B): a mass spectrum in the positive EI mode; (c): a mass spectrum in the NICI mode.**

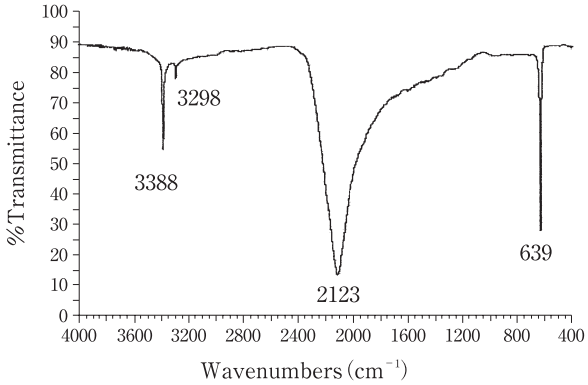
## Notes

a) Pure sodium azide ($NaN_3$) is white powder and well soluble in water; its p*K*a is 4.6. In acidic solution, it is converted into poisonous hydrazoic acid gas. The gas reacts with ferric chloride to produce a deep orange color, which can be used for a screening test for azide [12]. For informations, the infrared absorption spectrum and X-ray diffraction profile for sodium azide are shown in *Figures 3.3 and 3.4*, respectively.

b) In some of buffer solutions commercially available, sodium azide is included as a preservative. Before analysis of a test specimen, blank tests should be done to confirm the absence of azide in any material or reagent to be used for azide analysis.

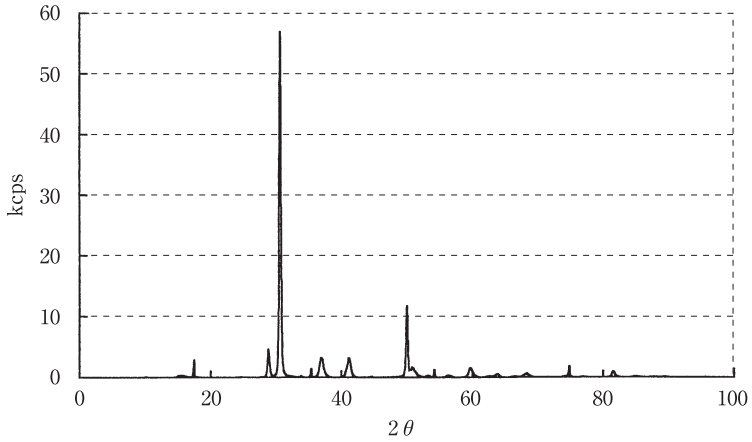
c) For the details of the Conway microdiffusion cell, please see Chapter II.1.3, Cyanide of this book.

**Figure 3.3**




**Infrared absorption spectrum of sodium azide.**

**Figure 3.4**




**X-ray diffraction profile for sodium azide.**

d) To realize sensitive analysis, an ion chromatograph system equipped with an autosuppressor is most desirable. In this system, the external mode, which uses pure water as regenerating solution, is adopted.

e) Since interfering ions may be eluted from a membrane filter, it should be sufficiently washed with ultrapure water before use.

f) Swelling particle polymer (200–400 mesh; polystyrene crosslinked with 1 % divinylbenzene) bound with the phosphonium salt. The commercially available product is the Cl type (0.90 mmol $Cl^-$/g. When only a compound with high nucleophilic reactivity like azide ion is targeted, the Cl type catalyst can be directly used. However, when anionic poisons with relatively low nucleophilic reactivity including cyanide ion are analyzed, it is preferable to convert the Cl type catalyst into the Br type according to the procedure described here. This is because the Br type catalyst acts to protect the derivatization reagent PFBBr from its

conversion into less reactive PFBCl by reaction with chloride ions being included in the biological matrix.

g) Azide ion is easily derivatized by nucleophilic displacement reaction with halogenated alkyl like PFBBr. The derivative produced is stable. Although PFBBr is a good derivatization reagent for GC/MS analysis in both positive EI and NICI modes, it is highly corrosive and lacrimatory and thus the handling of the undiluted solution of PFBBr should be carefully made inside a draft chamber.

h) By utilizing high nucleophilic reactivity of thiosulfate ion, PFBBr is converted into water-soluble and non-reactive pentafluorobenzyl-thiosulfate ion, which is removed at the steps iii)–v). PFB–Br + $^{-}$S-$SO_2$ – $O^{-}$→ PFB-S-$SO_2$-$O^{-}$ + $Br^{-}$. This reaction rapidly proceeds in a homogenous water-acetone system, but does not smoothly in the two- and tri-phase systems.

i) As ISs, Tetralin® [1], 1,3,5-tribromobenzene [3] and *N*-(2-trifluoro-methyl)benzyl pentafluoropropamine [2] were reported.

j) The specimen should be relatively clean with small amounts of impurities, such as lavage solution for a food and tea solution; as large as 4 mL volume of a specimen can be analyzed using an appropriate test tube with a ground-in stopper.

## References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