CASE REPORT

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Detection of Azide in Forensic Samples by Capillary Electrophoresis

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ABSTRACT: Azide salts are highly toxic compounds that have been difficult to detect in forensic samples. Here, anion analysis by capillary electrophoresis with indirect spectrophotometric detection was applied to detect azide in forensic specimens from two suicide victims. Gastric specimens from the victims were shown to have high azide concentrations; azide represented one of the major anionic components and no corresponding component occurred in normal gastric juice. Samples of blood and bile had low concentrations of azide near the limits of detection. The method described for azide analysis used simple steps for sample preparation and analysis time was less than 10 min per sample. It offers a simple and reliable method for detecting azide in biological fluids.

KEYWORDS: forensic science, forensic toxicology, azide, capillary electrophoresis, poisoning

Azide salts are toxic compounds widely used as preservatives in diagnostic reagents, as explosives, and as propellents in auto airbags. Azide salts have been the toxic agent in many poisoning cases (1–19). Death from ingesting less than 1 g of sodium azide has been described (10). The average lethal dose is considered to be 30 mg/kg body weight. Smaller doses can cause decreased blood pressure, severe headaches, breathlessness, vomiting, and neurological disorders. Azide binds to cytochromes and blocks oxidative metabolism similar to cyanide; it also is a hypotensive agent (20,21). Exposure can result from ingesting azide salts or inhaling volatile hydrazoic acid ($pK_a = 4.8$) formed from azide in acidic solutions (17). There have been no descriptions of what specimens are optimal for forensic investigation of azide poisoning or how to work up and analyze these specimens.

Two cases of probable suicidal ingestion of sodium azide led to investigation of methods for detecting and measuring azide in forensic and clinical samples. Although several methods for measuring azide are described (22–25) using titrimetry, ion chromatography, or derivatization followed by HPLC, these methods are not well-suited for analysis of azide in complex sample matrices such as blood, gastric contents, bile, urine, and vitreous humor. Recent studies have described improved methods for derivatization and analysis of azide derivatives by high performance liquid chromatography (18,19). The present study examined capillary electrophoresis as a means of measuring azide in forensic samples without the need for derivatization steps. Capillary ion analysis has previously been demonstrated as a versatile and high-resolution technique for ion analysis (26).

Methods

Sample Preparation

Postmortem samples of gastric contents, blood, and bile were prepared by dilution 10-fold with water followed by centrifugal ultrafiltration in Centricon-30 units (Amicon, Danvers, MA) to remove proteins and solids. Samples of wine, tea, and coffee from a scene of death were analyzed after dilution. Note that acidic solutions containing azide are a source of volatile hydrazoic acid. Thus, gastric juice from azide poisoning victims presents a potential source of poisonous gas and should be stored in well-sealed containers and handled in fume hood or area with suitable ventilation. Azide also can form explosive salts with copper or lead (for example in pipes) so that care must be taken for appropriate disposal of solutions containing azide.

Vapor microdiffusion was performed in sealed two-well Conway plates similar to the usual preparation of specimens for cyanide analysis. Two mL of sample was added to one well and adjusted to 0.1 M H_2SO_4. The other well contained 1 mL of 50 mM NaOH which trapped volatile acids such as hydrazoic acid released from the sample well. After 2 h at room temperature the base solution was collected, neutralized with acetic acid (acetate is a low mobility ion which migrates substantially slower than azide during capillary ion analysis), and diluted before analysis.

Capillary Ion Analysis

Analyses were performed in a fused silica capillary 75 μ internal diameter X 60 cm in length using a Quanta 4000^R system with data analysis using Millenium^R software (Waters Corp., Millford, MA). Separations at 20 kV used commercial reagents for anion analysis from Waters Corp. The electrolyte contained an electroosmotic flow modifier and 5 mM sodium chromate, pH 8.0, which

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served as a detector ion displaced by other anions. The electroendosmotic flow modifier is a cationic surfactant which reverses the electroendosmotic flow. Electroendosmotic flow and ion migration in the same direction toward the detector speeds analyses. Detection used a Hg lamp and 254 nm optical filter. Anions such as chloride and azide displaced the chromophore chromate and produced dips in absorbance proportional to their concentration. The absorbance signal was inverted to generate peaks rather than the actual troughs in absorbance and peak areas were determined with the Millenium software. Sample loading into the capillary was by 10 s gravity injection by raising the sample to a height of 10 cm over the capillary outlet. Sodium azide for use as a standard was purchased from Sigma Chemical (St. Louis, MO).

Results

Case Descriptions of Azide Overdose

A 25-year old female graduate student with a history of depression and a previous suicide attempt was found on the living room floor of her apartment with no pulse or respiration. A large volume of liquid vomitus was adjacent to the body. The decedent had been seen last 4 h earlier at dinner. She had been emotionally distressed. No suicide note was found. An empty bottle of spironolactone tablets and a bottle of sodium azide were noted at the scene as well as a glass of wine, empty cup of coffee, and cup of tea. Standard postmortem toxicological analysis of urine, blood, liver, and gastric contents by gas chromatography/mass spectrometry, immunoassays, and thin layer chromatography did not identify a toxic agent. Samples were positive for sertraline (blood level 0.1 mg/L), norsertraline, and caffeine and negative for alcohol and other drugs. Poisoning with sodium azide was suspected and this was confirmed by analysis of gastric contents by capillary electrophoresis, which detected a high concentration of azide, approximately 17 g/L.

A male graduate student who was an acquaintance of the decedent in the first case told a friend that he had ingested sodium azide. He expired about 1.5 h following ingestion despite rapid transport to an emergency room. His blood pressure at presentation of 118/70 mm Hg decreased over a course of 15 min to 76/29 mm Hg. His respiratory rate also fell from 20/min to 12/min. He was intubated, and efforts at resuscitation were unsuccessful. Toxicology studies of urine, blood, bile, and gastric contents were negative for ethanol and drugs of abuse. Capillary electrophoresis detected 0.65 g/L azide in gastric contents and low concentrations in blood and bile, near the limits of detection of the analysis (about 10-20 mg/L).

Azide Analysis

The physiological salt content of the forensic samples overloaded the capacity of capillary electrophoresis system. Sample overloading produced severe peak broadening and changes in migration time (not shown). Most samples required dilution 10 to 20-fold with water in order to achieve high-resolution analyses. Analyses of a gastric sample from the first suicide victim showed occurrence of a high concentration of an anion with a mobility the same as that of azide ion (Fig. 1, top panel). Due to the high concentration of this component, the analysis shown is for a specimen diluted 40-fold with water. This component was not observed in specimens of gastric juice (at a 10-fold dilution) collected post-mortem from other subjects for forensic analysis (Fig. 1, middle panel). Evidence supporting the identity of the component as azide was: 1) Migration times of the component were the same as azide run as a standard. 2) The putative azide peak increased in area and exhibited no shoulders when gastric juice from the suicide victim was spiked with sodium azide. 3) The component was volatile and could be trapped in an alkaline solution in a Conway microdiffusion plate and then detected by analysis by capillary electrophoresis. 4) Absence of the component in control post-mortem specimens. We have not observed any other anions that have the same mobility as azide in this analytical system. Nitrate and citrate are two anions expected to have mobility closest to that of azide (26).

A standard curve for azide concentration was linear over a broad concentration range (Fig. 2). Azide concentrations were calculated by relation to a standard curve and by evaluation of increases in peak area by spiking specimens. Both methods yielded similar re-

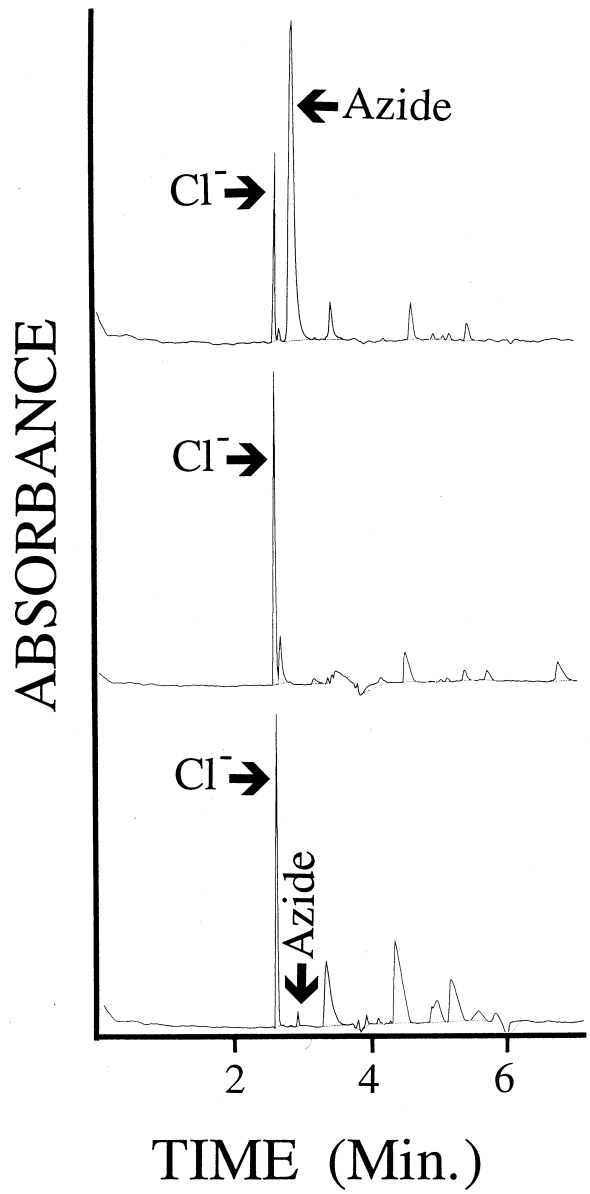

FIG. 1—*Electropherograms of anion analysis. Top panel: postmortem gastric juice (diluted 1:40 with water) from a suicide victim of azide overdose. Middle panel: normal postmortem gastric juice (diluted 1:10). Bottom panel: bile (diluted 1:10) of suicide victim. Peaks corresponding to chloride and azide are indicated.*

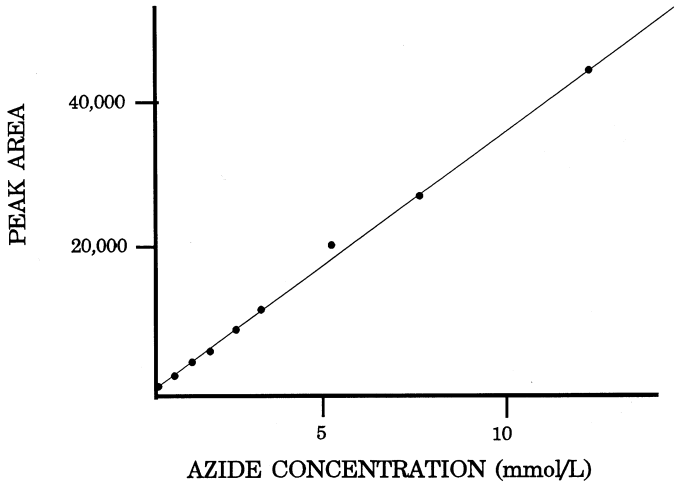

FIG. 2—*Calibration curve of peak area versus azide concentration.*

sults for the gastric analysis. The concentration of azide in gastric juice was determined to be 17 g/L for the first suicide victim and 0.65 g/L for the second. Thus, azide was one of the most abundant anions with a concentration of about 400 mmol/L and 15 mmol/L, respectively, in the two cases versus the concentration of about 100 mmol/L for chloride, the most abundant anion naturally occurring in gastric juice.

In blood and bile samples, small peaks approaching the limit of detection were observed with the mobility of azide. Analysis of bile at a 10-fold dilution is shown (Fig. 1, bottom panel). Peak area corresponded to an azide concentrations of about 40 mg/L in the original sample.

Samples of tea, wine, and coffee cup residue from the scene of the first suicide were analyzed in an attempt to identify the source of the azide that was ingested (data not shown). All of these specimens could be analyzed directly following dilution, but none of these samples contained detectable azide ion. It was concluded that the victim must have ingested sodium azide directly from a bottle of the dry reagent that was found at the scene.

Discussion

Detection of azide in forensic and clinical samples has been a difficult problem (22–25), and while this report was in preparation potential application of ion chromatography was described (27). Samples such as gastric contents and blood present a challenging sample matrix for analysis of azide due to their complexity, high protein content, and high concentration of other ions such as chloride. Use of capillary electrophoresis as described in this report offers an attractive means of detecting azide in complex biological specimens. Capillary ion analysis is well-established as a method of anion analysis (26). Specimen preparation is simple and analysis time could be as short as 5 min per specimen. Analytical cycle time includes 2 min to rinse the capillary between runs and a minimum of 3 min to allow migration of azide to the detector. The high salt content of biological fluids necessitated specimen dilution, but there was still sufficient sensitivity to detect azide in forensic specimens. Sensitivity might be enhanced by vapor microdiffusion of specimens into a basic solution. The method of capillary ion analysis could provide much higher sensitivity if azide could be separated from major inorganic ions such as chloride which necessitate dilution of the original specimen to avoid overloading. Opportunities for enhancing sensitivity by this approach were not fully explored in the present study, because additional sensitivity was not required for the cases in question. The method used in the present study was applicable to samples containing azide at concentrations greater than about 20 mg/L. This level of sensitivity was adequate for detecting azide in gastric contents following massive poisoning and in biological materials such as antisera and diagnostic reagents where sodium azide is commonly added as a preservative with a concentration of about 1 g/L. However, levels in other biological fluids remain at low levels that will often be below the limits of detection. Even in cases such as the two described here where substantial and lethal overdoses of azide were ingested, very low levels of azide were present in blood and bile. Binding of azide to cytochrome oxidases and to methemoglobin is likely to help keep levels of free azide low in blood. Results of the present study suggest that in cases of poisoning, gastric juice is the preferred specimen for detection of azide. The concentration of azide in gastric juice can be more than 100-fold higher than in other body fluids or tissues commonly used for forensic analysis.

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