DIONEX

# Application Note 172

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# Determination of Azide in Aqueous Samples by Ion Chromatography with Suppressed Conductivity Detection

## INTRODUCTION

Sodium azide is a white crystalline solid that is highly toxic when ingested or inhaled. The salt readily dissolves in water to yield the azide anion ($N_3^-$). Contact with water or acid causes release of hydrazoic acid ($HN_3$), a gas with a sharp, disagreeable odor that is considerably more toxic than the salt. Azide anion prevents the cells of the body from using oxygen, inhibiting the function of cytochrome oxidase by binding irreversibly to the heme cofactor.[1] Fatal doses occur with exposures of 700 mg (10 mg/kg), but exposure to smaller doses can cause eye and skin irritation, headache, nausea, shortness of breath, dizziness, blurred vision, low blood pressure, or kidney damage.

Sodium azide is best known as the chemical that triggers automobile airbag inflation. An electrical discharge triggered by automobile impact causes sodium azide to explode and release nitrogen gas inside the airbag. The nationwide production of sodium azide has increased to 6000 tons per annum, chiefly as a result of increased automobile airbag production. Sodium azide is also used as a chemical preservative in hospitals and laboratories, in agriculture for pest control, in pharmaceutical manufacturing, and in detonators and other explosives. Azide is also of interest to forensic investigators. Several people were poisoned in Japan in 1998 when azide was added into tea and orange juice. Ion chromatography was a key tool in diagnosing the cause of this poisoning.

Several methods are available to determine azide anion or hydrazoic acid. To determine sodium azide and hydrazoic acid in workplace atmospheres, an air sample is collected on an impregnated filter, desorbed into an aqueous sodium carbonate/bicarbonate solution, separated on an IonPac® AS9 column, and detected by UV absorbance at 210 nm (OSHA ID-211).[3] To determine azide anion in bodily fluids, Kruszyna described an approach based on trapping the hydrazoic acid sparged from an acidified sample, followed by ion chromatographic (IC) analysis of the trapping fluid.[4] Other methods include GC-MS of the pentafluorylbenzyl derivative,[5] and a micro diffusion extraction combined with spectrophotometry using the König cyanide reaction and ferric azide complex formation in conjugation with cerium azide oxido-reduction.[6]

This application note describes how to routinely monitor for azide anion in aqueous samples including water, food products, bodily fluids, and biological buffers by using a Reagent-Free™ IC (RFIC™) system. The azide anion is determined in 35 min by using a 4-mm IonPac AS15 column, isocratic potassium hydroxide provided automatically by an eluent generator, and suppressed conductivity detection. The IC method allows direct injection of the sample, avoiding laborious derivatization or sample preparation, and is highly sensitive, providing a detection limit for azide in reagent water of 50 µg/L.

## EQUIPMENT

A Dionex ICS-3000 chromatography system consisting of:

Dual Pump (DP)

Conductivity Detector (CDS)

Eluent Generator (EG)

AS Autosampler (AS)

Chromeleon® Chromatography Workstation with Chromeleon 6.7 Chromatography Management Software

Note: This application can be performed on any Dionex RFIC system.

## CONSUMABLES

EluGen® Hydroxide Cartridge (EGC II KOH) (P/N 058900)

ASRS® ULTRA II, 4 mm (P/N 061561)

Continuously Regenerating Anion Trap Column (CR-ATC) (P/N 060477)

Syringe filters (Gelman IC Acrodisc® 0.2 µm, PN 4483)

## CONDITIONS

| | |
|---|---|
| Columns: | IonPac AS15 Analytical, 4 × 250 mm (P/N 053940) |
| | IonPac AG15 Guard, 4 × 50 mm (P/N 053942) |
| Eluent: | 42 mM potassium hydroxide (KOH) |
| Flow Rate: | 1.2 mL/min |
| Temperature: | 30 ˚C |
| Injection: | 25 µL |
| Detection: | Suppressed conductivity, ASRS ULTRA II (4 mm), recycle mode |
| Power Setting: | 125 mA |
| Background Conductance: | 1–2 µS |
| Noise: | < 5 nS/min peak-to-peak |
| Backpressure: | ~2300 psi |
| Run Time: | 35 min |

## REAGENTS AND STANDARDS

Reagent grade water Type I, 18 MΩ-cm resistance or better, filtered through a 0.2-µm filter immediately before use

Seven Anion Standard II (P/N 57590)

Sodium azide (Sigma, P/N S-8032)

Sodium citrate (Sigma, P/N S-4641)

Fumaric acid (Fluka, P/N 47900)

Sodium chloride (JT Baker, P/N 4058-05)

Potassium chloride (Mallinckrodt, P/N 6858)

Sodium phosphate dibasic (Aldrich, P/N 21988-6)

Potassium phosphate monobasic (Fisher, P/N P285-500)

Sodium oxalate (Fluka, PN 71800)

Orange juice (Minute Maid® original, 100% Pure squeezed, P/N 0548 CT800)

Black tea (Lipton® 100% Natural Tea, P/N 83004654)

Green tea (Bigelow® P/N 44093EBE5)

Lyophilized citrated plasma (Sigma, P/N P9523)

## ELUENT SOLUTION

Generate the 42 mM KOH eluent on-line by using the EG Eluent generator system with an EGC II-KOH cartridge. Fill the plastic eluent reservoir with reagent water and maintain an inert helium atmosphere of 3–5 psi in the eluent reservoir.

Alternatively, prepare 42 mM NaOH by pipetting 2.92 g of 50% (w/w) aqueous NaOH from the middle portion of the reagent bottle into a 1-L volumetric flask containing about 900 mL of degassed reagent water. Do not shake the 50% (w/w) NaOH bottle or pipette from the top of the solution where sodium carbonate may have formed. Dispense the aliquot of NaOH below the surface of the water to avoid introducing carbon dioxide from the air into the eluent. Bring to volume with degassed reagent water, mix, and place the eluent in a plastic eluent reservoir under an inert helium atmosphere of 3–5 psi to minimize carbonate contamination.

*Note: Atmospheric carbon dioxide readily dissolves in dilute basic solutions, forming carbonate. Carbonate contamination of eluents can cause higher variance in retention time and lower sensitivity. Use of an EG system can improve retention time stability and sensitivity.*

## STOCK STANDARD SOLUTIONS

*Caution: Sodium azide is toxic. Consult the MSDS and wear proper protective gear to avoid inhalation,*

*ingestion, or skin contact with sodium azide. Sodium azide can react with heavy metal ions like copper, silver, or lead to form explosive metal azides. Do not pour concentrated azide solutions down the sink. Sodium azide can also explode when heated. DO NOT dry sodium azide in an oven.*

Prepare a 1000 mg/L stock standard solution of azide anion by dissolving 0.155 g of sodium azide in reagent grade water in a 100-mL volumetric flask. Bring to volume with reagent grade water and store the stock solutions in high-density polyethylene or polypropylene bottles at 4 ˚C.

### WORKING STANDARD SOLUTIONS

To prepare azide working standards, use a calibrated pipette to deliver the appropriate volume of the 1000 mg/L stock standard into a volumetric flask and bring to volume with reagent grade water. Method linearity was determined by diluting the stock solution to working standard solutions of 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05, and 0.025 mg/L.

To prepare mixed standards containing azide and other anions, combine appropriate volumes of the azide stock standard with the Seven Anion Standard II solution in a volumetric flask, and bring to volume with reagent water. Single-component anion standards may be used instead of Seven Anion Standard II.

### SYSTEM PREPARATION AND SETUP

Verify that the pump flow rate is within specifications and recalibrate if necessary. Verify that the conductivity cell constant is within specifications and recalibrate if necessary. Consult the pump or detector manuals for procedural details.

Install the EG and condition the EluGen II KOH cartridge as directed in the manual by running a gradient from 1 to 60 mM KOH in 20 min, then 60 mM for 40 min at 1 mL/min. (For instructions on installation and use, see the ICS-3000 IC system installation instructions, Document No. 065032).

Install and configure the autosampler. Use a calibrated sample loop in the full loop mode to obtain the best accuracy and precision. If you must make partial loop injections, program a sample volume that is less than half the volume of the installed sample loop, and program a cut volume of 8 µL. This injection procedure should provide peak area precision of <1% RSD.

Install a 1-mL sample syringe and set the syringe speed to 3. Enter the correct sample loop size and sample syringe volume in the AS Plumbing Configuration Screen. Refer to the ICS-3000 Ion Chromatography system installation instructions, Document No. 065032 for details.

Install a 4 x 50 mm IonPac AG15 and a 4 x 250 mm IonPac AS15 column. Make sure that the system pressure displayed by the pump is at least 2300 psi when 42 mM KOH is delivered at 1.2 mL/min so the degas assembly can effectively remove electrolysis gas from the eluent. If necessary, install backpressure coils supplied with the EG ship kit to adjust the system pressure to between 2300 and 2800 psi. Because the system pressure can rise over time, trim the backpressure coil as necessary to maintain system pressure under 3000 psi. Do not exceed 3000 psi or the degas assembly tubing may rupture.

Prepare the ASRS ULTRA II for use by hydrating the eluent chamber. Pump approximately 5 mL of regenerant reagent water through the Regen In port. Pump approximately 5 mL of reagent water through the Eluent In port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the ASRS ULTRA II in the recycle mode by following the Installation and Troubleshooting Instructions for the ASRS ULTRA II, Document No. 031956.

Equilibrate the column with 42 mM KOH eluent for 60 min, and analyze a system blank of reagent water. A well-equilibrated system should have a conductance between 1–2 µS and peak-to-peak noise of <5 nS/min.

Inject a Seven Anion standard spiked with 10 mg/L azide. The column is equilibrated when two consecutive injections of the standard produce the same retention time for azide. Confirm that the resulting chromatogram resembles the chromatogram of the 10 mg/L standard shown in Figure 1.

### INTERFERENCE STUDIES

To determine whether other anions interfere with azide determination, single-component standard solutions containing 10 mg/L of the following anions were injected: fluoride, chloride, nitrite, sulfate, oxalate, fumarate, nitrate, adipate, and bromide. Also, a mixed standard was injected containing 10 mg/L each azide, fumarate, and phosphate, 5 mg/L each chloride, nitrite, sulfate, oxalate, bromide, and nitrate, and 1 mg/L fluoride.

## SAMPLES

Samples can be directly injected after minimal sample preparation. The samples must be diluted (1:10 or 1:50 in reagent water) and filtered to remove particulates, otherwise, overloading of the AS15 column by the matrix ions will cause the azide peak to appear shorter and broader. The azide peak will be harder to reliably integrate, and low concentration detection limits cannot be achieved.

For this application, phosphate-buffered saline solution (PBS) was prepared by adding 1.00 g NaCl, 0.025 g KCl, 0.18 g $Na_2HPO_4$, and 0.03 g $KH_2PO_4$ to about 50 mL reagent grade water in a 100-mL volumetric flask, swirling to dissolve, and bringing to volume with reagent grade water. The PBS was diluted 50-fold with reagent grade water and filtered through a syringe filter before injection. For spike recovery measurements, the PBS was spiked with 10 mg/L sodium azide before dilution and filtration.

Green tea and black tea were prepared by steeping a teabag in about 100 mL of hot reagent grade water for 10 min. After cooling, the infusion was diluted 10-fold with reagent grade water and filtered before injection. For spike recovery measurements, the tea was spiked with 10 mg/L sodium azide before dilution and filtration.

Orange juice was diluted 10-fold with reagent grade water and filtered before injection. For spike recovery measurements, the orange juice was spiked with 10 mg/L sodium azide before dilution and filtration.

Human urine was diluted 10-fold with reagent grade water and filtered before injection. For spike recovery measurements, the urine was spiked with 10 mg/L sodium azide before dilution and filtration.

Lyophilized, citrated human plasma was reconstituted by adding 10 mL of reagent water to one vial and gently mixing to completely dissolve the powder. This reconstituted plasma was then diluted 10-fold with reagent grade water and filtered prior to use. For spike recovery measurements, the plasma was spiked with 10 mg/L sodium azide before dilution and filtration.

## RESULTS AND DISCUSSION

Table 1 summarizes the calibration data for typical calibration curves obtained by injecting calibration standards at 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, and 10 mg/L. The calibration curve in reagent grade water is linear, with a correlation coefficient of 0.9998. Figure 1 shows an ion chromatogram of a 10 mg/L azide standard along with several commonly occurring anions, obtained by using the optimized conditions described above.

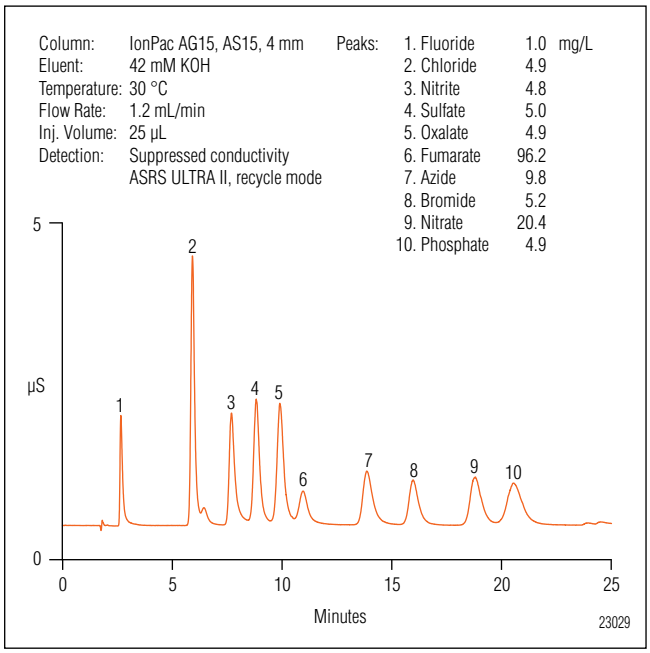


*Figure 1. Determination of a 10 mg/L azide standard along with several commonly occurring anions.*

**Table 1. Linear Range and Detection Limits of Azide in Various Matrices**

| Matrix | Range (mg/L) | $R^2$ | MDL Standard (µg/L) | % RSD | *Calculated MDL (µg/L) |
|---|---|---|---|---|---|
| Reagent Water | 0.025 – 10 | 0.9999 | 100 | 9.8 | 51 |
| Black Tea | 0.025 – 10 | 0.9989 | 500 | 13.0 | 140 |
| Green Tea | 0.025 – 10 | 0.9996 | 500 | 6.1 | 68 |
| Orange Juice | 0.025 – 10 | 0.9940 | 500 | 12.3 | 155 |
| PBS | 0.025 – 10 | 0.9924 | 500 | 6.3 | 87 |
| Urine | 0.025 – 10 | 0.9995 | 500 | 9.5 | 133 |
| Plasma | 0.025 – 10 | 0.9978 | 250 | 2.2 | 34 |

*The MDLs were calculated as MDL = (t) × (SD) Where t=Student's t value for a 99% confidence interval and a standard deviation estimate with n – 1 degrees of freedom [t = 3.14]

The method detection limit (MDL) for azide was determined by making seven injections of a low-level solution fortified with azide at a level that yielded a signal-to-noise of about 6–9. The concentration values determined from the calibration curve were used to calculate the MDL.

The MDL for azide in reagent grade water was determined by making seven replicate injections of reagent water fortified with azide at 100 ppb. The calculated MDL from this work is given in Table 1. The MDL is defined as the minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. It is basically a measure of the precision of preparing and analyzing low-level samples according to the method. The MDL for azide in reagent water obtained by this method is 50 µg/L.

Azide was determined in several different matrices that varied in ionic strength and the presence of possible interferences. Some of the samples exemplify matrices of forensic interest, for example orange juice, tea, urine, and plasma.

For each matrix, precision, recovery, and freedom from interferences were measured. Precision and recovery were measured by spiking 1 mg/L azide into seven matrices; reagent water, black tea, green tea, orange juice, PBS, urine, and plasma. Recovery for all matrices was greater than 80%. Precision varied from 2 to 8% for all the matrices. The results are shown in Table 2.

Both green tea and black tea were diluted 1:10. Figure 2 shows the determination of 10 mg/L of azide spiked into green tea. Fluoride, chloride, sulfate, and oxalate anions were observed. Figure 3 shows the determination of 10 mg/L of azide spiked into orange juice. Orange juice contains phosphate, sulfate, fluoride, and fumarate. These compounds do not interfere with azide.

**Table 2. Recovery of Azide from Various Matrices**

| Matrix | Amount Added (mg/L) | Recovery (%) (n=3) | Precision (RSD) (n=3) |
|---|---|---|---|
| Reagent Water | 1 | 100 | 4.4 |
| Black Tea | 1 | 91 | 5.4 |
| Green Tea | 1 | 104 | 1.9 |
| Orange Juice | 1 | 87 | 8.0 |
| PBS | 1 | 98 | 4.1 |
| Urine | 1 | 108 | 3.7 |
| Plasma | 1 | 117 | 2.2 |

Sodium azide is commonly used as a preservative in aqueous laboratory reagents and biological fluids. Azide was spiked into PBS and plasma in order to help characterize sodium azide in some fluids commonly used in biological laboratories. PBS contains large amounts

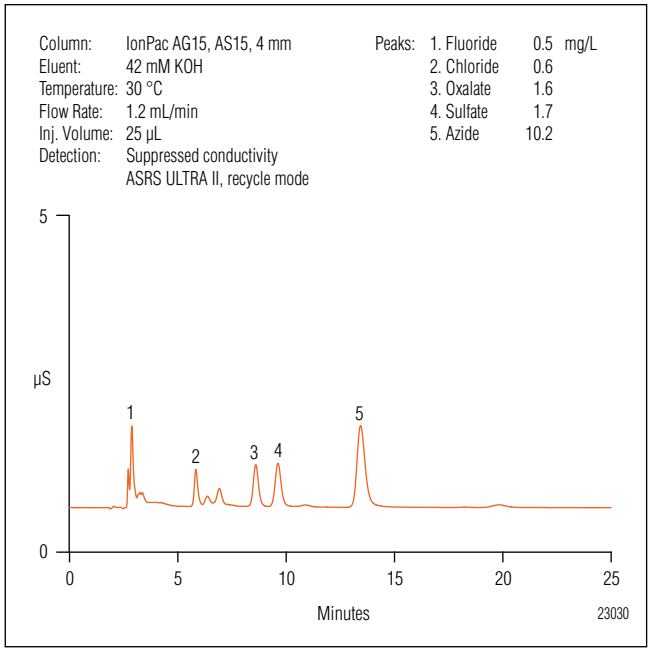


*Figure 2. Determination of 10 mg/L azide spiked into 10-fold diluted green tea.*

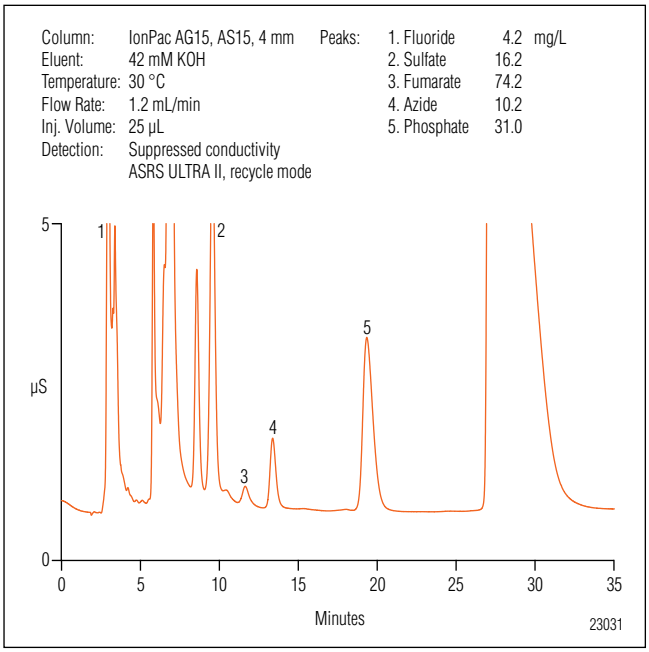


*Figure 3. Determination of 10 mg/L azide spiked into 10-fold diluted orange juice.*

of chloride and phosphate that can interfere with the integration of azide if it is not diluted. Figure 4 shows the analysis of dilute PBS samples spiked with azide and reveals that phosphate and chloride do not interfere with the determination of azide.

Forensic analysis may require that urine and plasma be analyzed for azide. Figure 5 shows the determination of 10 mg/L azide spiked into urine. None of the component anions interfere with azide quantification. Figure 6 shows the determination 10 mg/L azide spiked into citrated plasma. None of the component anions interfered with the integration of azide under the conditions used for the study.

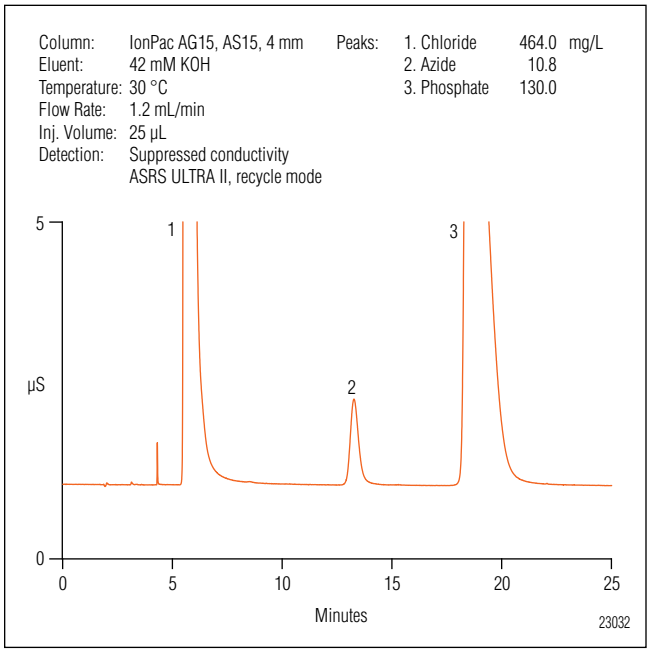


*Figure 4. Determination of 10 mg/L azide spiked into 50-fold diluted PBS.*

## INTERFERENCES

None of the anions tested, at concentrations up to 10 mg/L, interfered with the determination of azide. Figure 1 shows that azide is well resolved from all of these anions under the conditions shown.

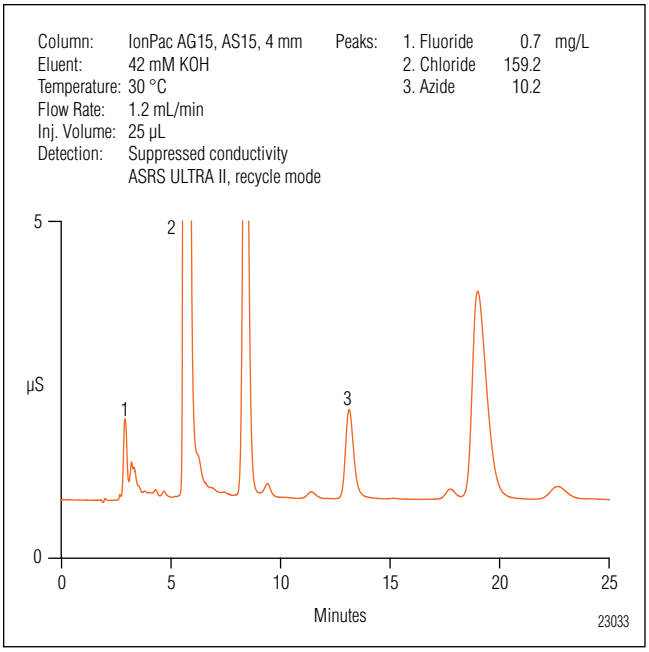


*Figure 5. Determination of 10 mg/L azide spiked into 10-fold diluted urine.*

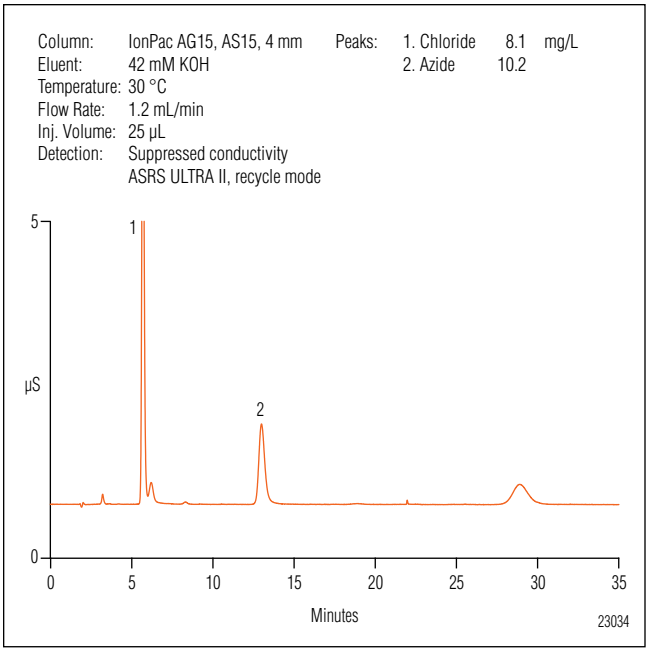


*Figure 6. Determination of 10 mg/L azide spiked into 10-fold diluted plasma.*

## SUGGESTIONS FOR BEST PERFORMANCE

These results were obtained by using a 4-mm AG15/AS15 column set with a 25-µL injection of sample. Peak area precision and accuracy depend on autosampler performance. Replace the water in the flush reservoir daily with freshly filtered and degassed water. Inspect the AS daily for bubbles in the sample syringe or its tubing. Purge to remove any bubbles by following the instructions in the AS manual.

Strongly retained compounds from injected samples can accumulate on the column and degrade its performance. Signs of a fouled column include loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. To remove low valency, hydrophilic contaminant ions, flush the column with a 0.42 M hydroxide solution (i.e., a 10X concentrate of the most concentrated eluent used in the application). To remove high valency, hydrophobic contaminants, flush the AS15 column with 0.2 N HCl in 80% $CH_3CN$. (For more information on column troubleshooting and cleanup, see the Installation Instructions and Troubleshooting Guide for the IonPac AS15 Analytical Column, Document No. 031362.)

Some samples contain particulates that will plug the column and increase the backpressure. Use a guard column to protect the analytical column; change the guard column if such a sample causes a sudden increase in total backpressure to greater than 3000 psi.

*Caution: Sodium azide is toxic. Consult the MSDS and wear proper protective gear to avoid inhalation, ingestion, or skin contact with sodium azide. Sodium azide can react with heavy metal ions like copper, silver, or lead to form explosive metal azides. Do not pour concentrated azide solutions down the sink. Sodium azide can also explode when heated. DO NOT dry sodium azide in an oven.*

## SUMMARY

The resulting MDL for azide in several matrices ranges from 51 µg/L in reagent grade water to 155 µg/L in orange juice. Calibration is linear over the range of 0.025–10 mg/L in reagent grade water as well as other matrices and quantitative recoveries were obtained for azide spiked at 1 mg/L concentrations. The method provides acceptable performance, in terms of peak shape and recovery, in the presence of high amounts of chloride, sulfate, oxalate, and phosphate.

## REFERENCES

1. *Fed. Regist.* **1989,** *54* (12), 2540.
2. Chang, S.; Lamm. S. H. Human Health Effects of Sodium Azide Exposure: a Literature Review and Analysis. *Int. J Toxicol.* **2003**, *22* (3), 175–186.
3. Ku, J. C. *Sodium Azide and Hydrazoic Acid in Workplace Atmospheres*. OSHA_SLTC Method ID-211. U.S. Department of Labor, Occupational Safety and Health Administration, 1992.
4. Kruszyna, R.; Smith R. P.; Kruszyna, H. Determining Sodium Azide Concentration in Blood by Ion Chromatography. *J. Foren. Sci.* **1998**, *43*, 192-194.
5. M. Kikuchi, M. Sato, T. Ito and M. Honda.Application of a New Analyical Method Using Gas Chromatography and Gas Chromatography-Mass Spectrometry for the Azide Ion to Human Blood Samples of an Actual Case. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2001,** *752* (1), 149–157.
6. Tsuge,K.; Kataoka, M.; Seto, Y. Rapid Determination of Cyanide and Azide in Beverages by Microdiffusion Spectrophotometric Method. *J. Anal. Toxicol.* **2001**, *25* (4), 228–236.





LPN 1831 PDF 09/16