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Original Article

Assessment of Significant Anions of Nitrite, Nitrate, Fluoride, Chloride, Sulfate and Phosphate in Mineral and Drinking Bottled Waters and Their Roles in Contamination

Sima Djam[1], Mostafa Najafi[2], Seyyed Hamid Ahmadi[3], Shahram Shoeibi[4*]

1- Department of Chemistry, South Tehran Branch, Islamic Azad University, Tehran, Iran

2- Department of Chemistry, Imam Hossein University, Tehran, Iran

3- Department of Environmental Analytical Chemistry, Chemistry & Chemical Engineering Research Center of Iran, Tehran, Iran

4- Food and Drug Laboratory Research Center (FDLRC), Iran Food and Drug Administration (IFDA), Ministry of Health and Medical Education, Tehran, Iran

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ABSTRACT

Background and Objectives: Mineral and drinking waters are categorized bottled waters in the national standard of Iran and use of bottled waters is most linked to their health issues. Anions such as nitrite, nitrate, fluoride, chloride, sulfate and phosphate are important in assessing source and time of contamination in the purification water industry. National and international standards have been set and justified for the permissible levels for the highlighted anions.

Materials and Methods: Totally, 168 minerals and drinking bottled water samples from the Iranian markets were collected under ideal sampling conditions and analyzed using ion chromatography (IC) featured with a conductivity detector.

Results: Results showed that four anions, including nitrate, fluoride, chloride and sulfate, in all samples were detectable with mean values of 0.131, 6.77, 25.109 and 20.97 ppb, respectively, which were lower than the maximum standard levels. Nitrate and phosphate were not detected in 86% of the samples and included very low levels in other samples.

Conclusions: It can be concluded that the anions are reliable sources of contamination and their monitoring is necessary for the product safety and consumer health.

Keywords: Bottled water, Anions, Product safety, Ion chromatography

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Introduction

Bottled water has been used widely in recent years. Bottled water in most countries is originated from spring water or pure underground water with no changes. The European Union (EU) states that bottled water includes natural mineral water, spring water and other drinking water (1). Mineral water includes bottled water for human use with no additives (2). According to the definition of Iran National Standard Organization (ISIRI), bottled water may include natural or added minerals and CO_2. However, no added sweetener flavors are permitted. Bottled water sources include groundwater originating from aquifers such as artesian wells, springs, wells and subterranean canals. In addition, water supply networks and normal glaciers can be used for bottled water. Based on ISIRI, bottled water is classified into two classes; drinking and mineral bottled waters that must be free from any external materials such as sand, hair and insects (3). There are specific rules for mineral and drinking bottled waters (4). Moreover, people would like to ensure of bottled water quality and safety. Contaminations may be seen in bottled water such as risky microbial and inorganic and organic chemical contaminations (5). US National Primary Drinking Water standards postulate a maximum contaminant level (MCL) for inorganic anions such as nitrite, nitrate and fluoride. The MCLs are published for minimizing possible health risk effects resulted from the intake of anions in water supplies (6, 7).

The anion toxicity levels (nitrates, nitrites, sulfate, fluorine, phosphate and chloride) are vital for the environmental sustainability and human health. When the disinfection process is carried out, chlorine may react with effluent organic matters and utilize iodine and bromide ions to form various disinfection derivatives (8–10). Nitrites and nitrates are because of the run-off nitrogenous

***Address for correspondence:** Shahram Shoeibi, Associate Prof, Food and Drug Laboratory Research Center (FDLRC), Iran Food and Drug Administration (IFDA), Ministry of Health and Medical Education, Tehran, Iran. E-mail address: sh.shoeibi@fda.gov.ir

fertilizers released from the sewage sites and septic tanks. Nitrates can cause methaemoglobinemia (11). Chlorination is a conventional disinfection way in treatment of drinking water (12). Fluoride is a necessary trace element for humans. However, high concentrations of fluoride are toxic and may result in death since they block various enzymes (13). Fluorine is majorly found in rocks and minerals in the lithosphere (14). Fluorine is a fundamental micronutrient (as fluoride F^-) for humans, especially for preventing dental caries and osteoporosis treatment. Extreme and long-term intake may result in fluorosis, tooth enamel demineralization and softening of the bones (15).Nitrites can react with secondary and tertiary amines creating highly carcinogenic nitrosamines. The water source contamination with NO_3^- is a growing concern. The nitrate sources in drinking water are frequently found in septic systems and wastewater treatment plants, agricultural fertilizers and landfills (16). For example, nitrite (NO_2^-) may interact with hemoglobin, forming methemoglobin by Fe^{+2} oxidation to Fe^{+3}, that interrupts blood ability to transport oxygen (17, 18). The NO_3^- reacting with amines generates N-nitrosamines that are possible mutagens, carcinogens and teratogens (19). Sulfate is the most common anion in water, following chloride and bicarbonate. Because sulfate creates $MgSO_4$ and $CaSO_4$ sediments in thermal water, it is necessary to keep the sulfate level low in this water (20).

Due to the oscillation of over-fertilization, agricultural land and municipal or industrial resources, increases of phosphor entry to natural waters create adverse effects as eutrophication and exponential algal bloom include harmful effects for the fish. It is believed that high phosphate levels in the water environment are created by the presence of essential nutrients in the environment for biological generation (21). Several analytical approaches have been used to assess inorganic anions in drinking waters, including capillary ion electrophoresis (22, 23) and ion chromatography (24–26). Of these approaches, ion chromatography is the most extensively used method, suggested by USEPA as an official approach for analyzing drinking water samples in USA (27). Small et al. presented the first approach for quantitative determination and separation of inorganic ions through high-performance liquid chromatography (HPLC) (28). This method, known as ion chromatography (IC), uses a combined suppressor column and analytical column to decrease the mobile-phase electric conductivity in conductometric detection (29). In this study, most drinking and mineral bottled water brands were collected from Iranian markets and analyzed for six selected anions using ion chromatography. These anions were routinely controlled for tap water and water network, but the present study is novel in Iran product safety.

Materials and Methods

Apparatus and Parameters

The IC system included Metrohm Series 7000 and Metrohm 732 Conductivity Detector (Metrohm, Switzerland). Chromatographic separations were carried out using Dionex IonPac AS23 (250 × 4.0 mm) using AG23 Pre-column (50 × 4.0 mm). Buffer solution of 4.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ were developed by dissolving 0.445 g Na_2CO_3 in 1000 ml of double distilled water (DDW) as well as 0.084 g NaHCO3 in 1000 ml of DDW, respectively. The buffer solution was degassed before filtered through 0.45-µm nylon membrane filters. The flow rate was fixed to 1.1 ml/min at 25 ˚C. The injection loop volume was 20 µl and the run-time was 27 min.

Materials and Preparation

Sodium bicarbonate and sodium carbonate were purchased from Merck, Germany. The Ultra-Pure ELGA Pure Lab (UK) generated DDW (18 MΩ cm at 25 °C). Class A volumetric flask were used in sample procedures. The standard stock solutions of fluoride, nitrate, chloride, phosphate and sulfate were made at 1000 mg/L concentrations in DDW and nitrite was prepared at 100 mg/L concentration; all stored at 4 °C until use. Moreover, working standard solutions were prepared by diluting the stock standard solution with the DDW daily.

Optimizing Chromatographic Condition

The optimal analytical condition was experimented using various column types, mobile phase concentrations, flow rates and loop volumes. Two columns of Metrohm Metrosep A Supp 5 (250 mm × 4.0 mm) and Dionex IonPac AS23 (250 mm × 4.0 mm) were used. Considering fluoride, nitrate, chloride, phosphate, sulfate and nitrite peaks, Dionex IonPac AS23 offered increased resolution chromatography and sharpness. The buffer composition concentration and loop volume were assessed as well and the optimal conditions were selected for these parameters.

Calibration Curve

Fluoride solution was prepared at 1 mg/L and used for the development of five various concentrations (0.31, 0.62, 1.25, 2.50 and 5.00 µg/L). Chloride solution was prepared at 2 mg/L and used to generate five various concentrations (0.62, 1.25, 2.5, 5, 10 µg/L). The Nitrate solution was prepared at 1 mg/L and used to generate five various concentrations (0.31, 0.62, 1.25, 2.5 and 5). The Nitrite solution was prepared at 1 mg/L and used for the generation of five various concentrations (0.03, 0.06, 0.12, 0.25 and 0.5). The Phosphate solution was prepared at 0.2 mg/L and used for the generation of five various concentrations (0.06, 0.12, 0.25, 0.5 and 1). Sulfate solution was prepared at 2 mg/L and used to generate five various concentrations (0.62, 1.25, 2.5, 5 and 10). Each concentration was injected into the IC three times per day on three various days.

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Table 1. The optimized parameters of ion chromatography for the analysis of highlighted anions

Column	Mobile Phase (mM)	Flow Rate (ml/min)	Loop Volume (µl)	Detector	Retention Time (min)
Dionex AS23/ Guard AG23	Na_2CO_3:$NaHCO_3$ 4.2:1	1.1	20	Conductivity Metrohm 732	Fluoride: 6.13 Chloride: 6.56 Nitrite: 10.01 Nitrate: 15.25 Phosphate:19.80 Sulfate:22.98

Sample Preparation

In total, 167 samples of drinking and mineral bottled waters were collected from active manufacturers in 500 and 1500-ml volumes with three bottles from each sample and sent to the General Laboratory of Food and Drug Control of Iran, Ministry of Health and Medical Education, Tehran, Iran. These brands covered Iranian markets at the time of sample collection, involving nearly 80% of bottled water production. Other companies were temporally producing or not producing bottled water at that time.

Results

The anions were analyzed using IC and the linearity of the analytical method was verified by drawing calibration curves (Figure 1). Since optimizing analysis factors, standard dilutions of anions nitrite, nitrate, fluoride, chloride, sulfate and phosphate were prepared using the stock solutions, analyzed and linearity between the concentrations and responses were calculated based on area under curve (AUC). The calibration curves that IC analyzes are shown in Figure 1. Regression coefficients (r^2) of the highlighted analytics were achieved at high acceptable quantities. To achieve accurate and competent repeatability and reproducibility, acquired data were triplicated and repeated during the day with appropriate time intervals on three various days (inter and intra-day).

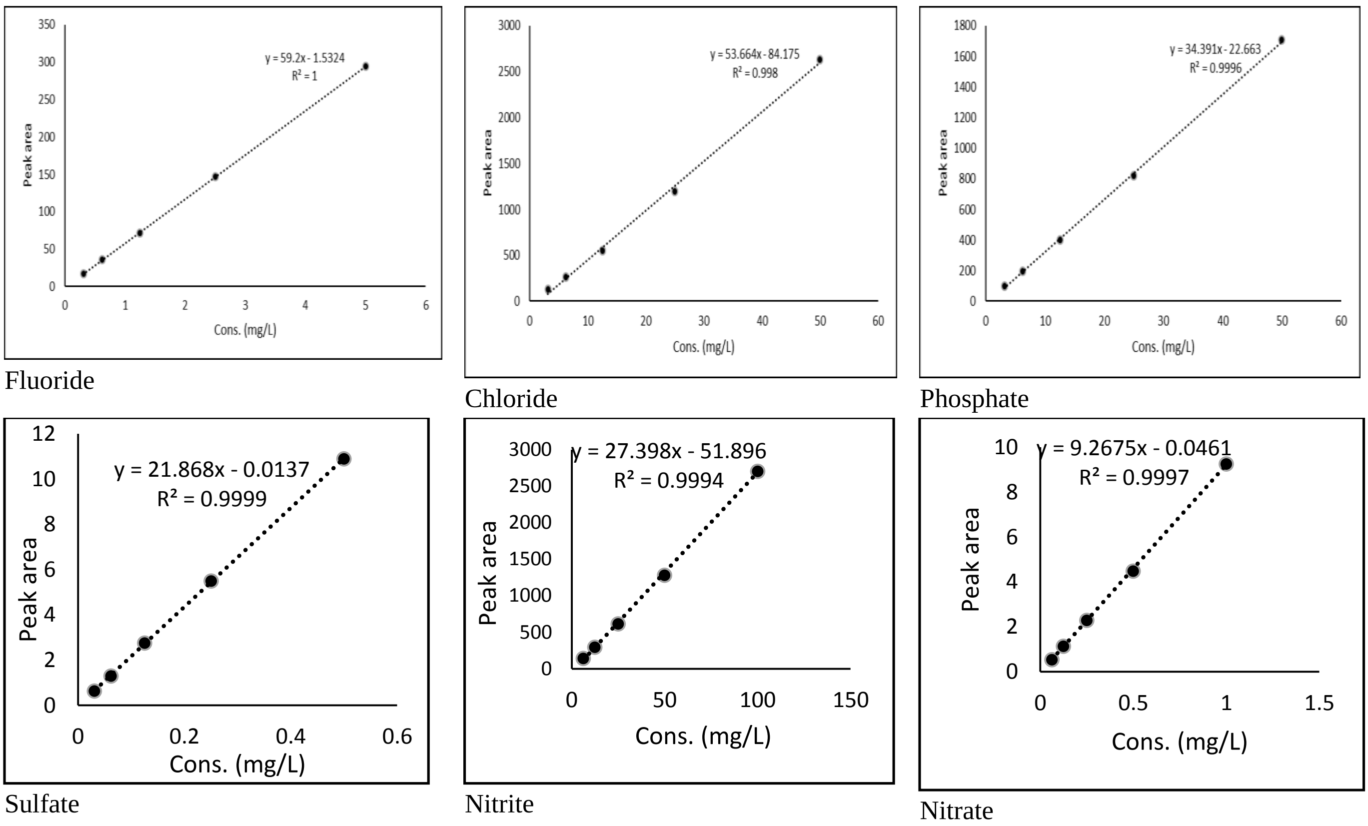

Figure 1. Calibration curve of the highlighted anions (Cl^-, F^-, SO_4^{2-}, PO_4^{3-}, NO_3^- and NO_2^-)

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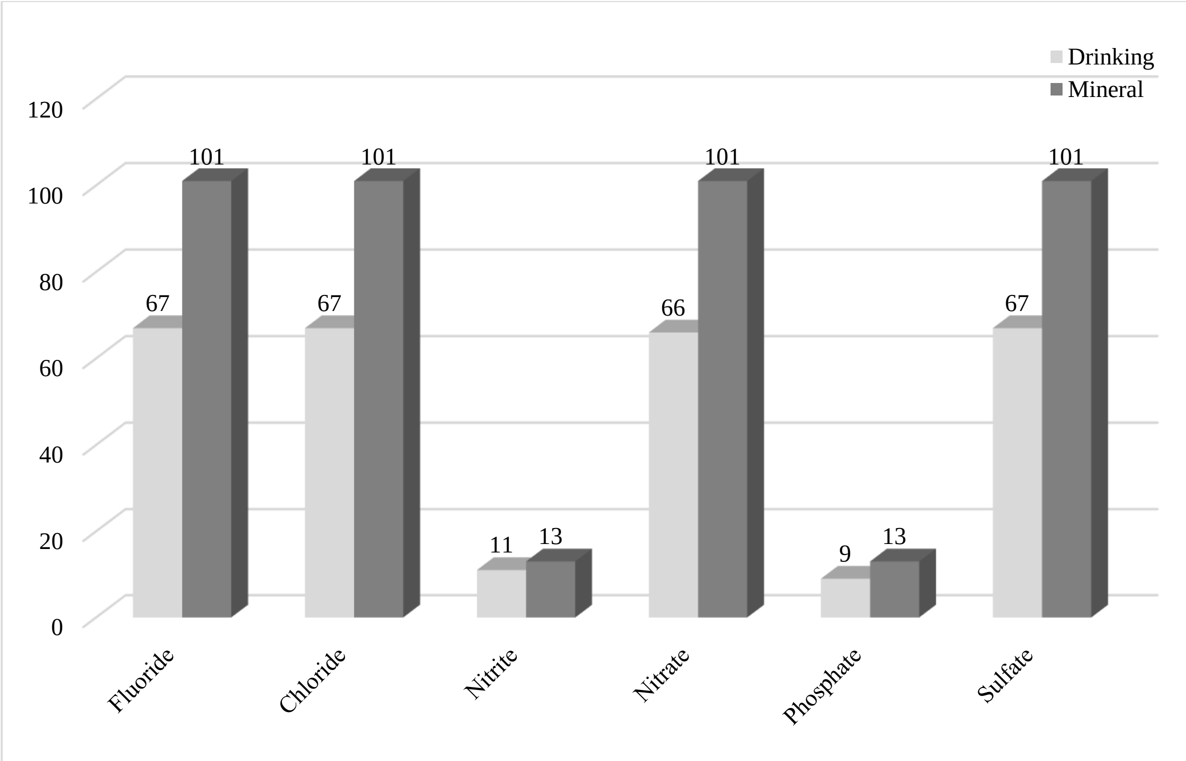

Figure 2. Number of the highlighted anions detected in mineral and drinking bottled waters

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Nitrite, nitrate, fluoride, chloride, sulfate and phosphate contents of the 168 bottled water samples, including mineral and drinking water samples, were analyzed. Samples belonged to the biggest manufacturers of Iran. However, some producers occasionally produce, depending on the seasons. Figure 2 shows the number of anions detected by IC. All bottled water samples included fluoride, nitrate, sulfate and chloride in detectable measures with a mean of 0.131, 6.77, 25.109 and 20.97 ppb, respectively, which were lower than MRL set by ISIRI. These results have shown that sources of water in manufacturing locations have not been contaminated for a long time. Nitrite and phosphate have not been detected in nearly 86% of the bottled water samples, and the average measures of these parameters in bottled water samples were 0.019 and 1.52 ppb, respectively.

Discussion

Ion chromatography, a flexible and powerful method for simultaneously study of numerous ions in water and food samples, is often used to assess inorganic and organic anions and cations. Furthermore, the technique lacks toxic chemicals, which are usually needed for wet chemical techniques. Ion chromatography includes a wide variety of uses, substantial accuracy and precision, well-developed hardware, several detection options, high selectivity, separation efficiency, good toleration to sample matrices and low cost of daily uses. Based on the national and international standards, these anions can be excellent and reliable indices for the judgment of contamination period. Most of these standards use results of the interested anions for the interpretation of hygiene and consumables bottled waters. Results of similar studies that analyzed anions in bottled waters were similar to results of the current study. In 2015, Daraei et al. studied ten brands of mineral water, while in the present study, brands were countryside covering and samples were selected based on two various definitions of mineral and drinking waters, defined by national standards. Furthermore, sample collection was carried out during the entire year (30). Knock et al. in 2018 studied European bottled water for anion and cation ingredients. Levels of the assessed anions were different in comparison with the levels of declared labels, but similar to their standard levels. They concluded that reasons the method and duration of water storage, technology of water preparation and use of analytical methods without validation might explain the differences (31). It is thus concluded that routine control of anions in bottled waters is necessary for the safety of products and consumer health. Although in most studies, the key anion levels were reported less than those of the established standards.

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