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RESEARCH

Evaluating boron levels in Turkish mineral waters: a comparative study of three analytical techniques

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Abstract Turkey is abundant in natural mineral water sources, thanks to its location on the Alpine-Himalayan belt. Natural mineral water is drinking water characterized by its natural mineral, trace elements, and carbon dioxide content. Because of quite insufficient data, the boron content in bottled natural mineral waters in Turkey was analyzed by three different methods and compared: inductively coupled plasma mass spectrometry technique, carminic acid, and azomethine-H methods, in this study. The boron levels in mineral waters ranged from a minimum of 0.05 mg/L to a maximum of 8.61 mg/L. It was also safe by the upper limit level estimated by the World Health Organisation. As boron plays a beneficial role in human physiology, consuming natural mineral water may offer a positive contribution to public health by supporting boron intake in our country. The other outcome of our research was that the spectrophotometric carminic acid method can yield results similar to those obtained using the inductively coupled plasma mass spectrometry technique since the boron level of Turkish mineral water was within the limits level of the carminic acid method. However, the result of the azomethine-H method was found not to be suitable. Cross-sensitivity with other elements in mineral water might have caused this.

Keywords Boron · Natural mineral water · ICP-MS · Carminic acid · Azomethine-H

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Introduction

Natural mineral waters are rich in minerals and trace elements. They are originated from underground waters. Surface waters such as rain, and snow seep into the ground and are stored for several years. By the effect of temperature and pressure, stored water can naturally move toward the surface along fractures and cracks, or it can be drilled. Since Turkey is a part of the Alpine-Himalayan belt, it has many natural mineral water resources (Altikulac et al., 2022; Glazier, 2009).

Boron is a metalloid with atomic number 5, molecular weight 10.81 g/mol, and symbol B. In nature, it does not occur as an element, instead, it forms compounds with elements like oxygen, and sodium (Uluisik et al., 2018). Different B compounds are used in many areas like cleaning, energy, glass, ceramic, and agricultural industries. It has been also suggested that boron is essential for human physiology. It plays an essential role in bone mineralization, immunity, mineral and hormone metabolism, energy metabolism, reproductivity, and brain function (Biţă et al., 2022; Khaliq et al., 2018; Uluisik et al., 2018).

People get boron mainly from foods and drinking water. Since it is a mineral component of the Earth's crust, it also accumulates in mineral waters. Recommended dietary allowance (RDA) levels have not been set for boron yet due to insufficient data. However, according to the World Health Organization (WHO), the tolerable upper intake level for boron is 0.4 mg/kg per day. It equals about 28 mg/day for a 70 kg person (WHO, 1996; Seidel et al., 2021).

This study examines the boron content in Turkish mineral waters, an area with limited data. It compares three methods (Inductively Coupled Plasma-mass spectrometry- ICP-MS, carminic acid, azomethine-H) for boron detection, a novel comparison in this context. Considerations include equipment cost and safety, proposing alternatives to expensive techniques like ICP-MS. While ICP-MS offers greater sensitivity and lower detection limits, its high cost may pose affordability challenges. Spectrophotometric analyses using carmine or azomethine-H dyes to produce colored complexes offer alternative methods. Studies indicate that the carminic acid method, while less sensitive to interferences, poses risks due to chemicals like concentrated sulfuric acid. The study evaluates the azomethine-H method, which does not require concentrated acids but may face interference from other elements (Carrero et al., 2005; Floquet et al., 2016). It also explores boron intake through natural mineral water consumption, providing health insights. Overall, the research offers a comprehensive analysis of boron content in Turkish mineral waters, comparing various analytical methods, addressing practical and safety concerns, and discussing broader implications for public health.

Material and method

Natural mineral waters

Bottled mineral waters of different brands were purchased from markets in Turkey. Twenty-one different brands were included and five samples from each brand were used. Samples were numbered from 1 to 105 to keep the brands anonymous. According to the label information of each sample, mineral waters originated from the cities of Afyonkarahisar, Ankara, Burdur, Bursa, Eskişehir, Giresun, Karabük, Erzincan, Manisa, Niğde, and Trabzon were used (Fig. 1). All of 105 samples was divided into three parts for the ICP-MS technique, carminic acid, and azomethine-H methods. To prevent contamination, disposable and boron-free materials were used, and all reagents met the analytical grade.

ICP-MS technique

AT.05.043 determination of multi element as a method was created by combining EPA 6020A (Rev 1–2007) and EPA 200.8 (Rev 5.4–1994) methods as a reference. For the ICP technique, mineral water samples were diluted 5 times with 1% nitric acid (Merck, Darmstadt, Germany) solution (4 volumes of 1% nitric acid + 1 volume of water sample). After each sample was filtered, it was transferred to the automatic sampling unit of the ICP-MS (Thermo Scientific ICAP RQ). For each analysis, at least three replicates were performed, average values were determined, and data analysis was performed. Six standard solutions with boron concentrations ranging from 0.01 to 2.0 mg/L were prepared using 1000 µg/mL stock boron standard solution (Supelco 1.11355.0100) and the calibration curve was plotted with the results of boron mass 10 (B10). The operational settings for ICP-MS were as follows: Plasma Power was set to 1550 W, Nebuliser gas flow rate was set to 1.2 L/min, Auxiliary gas flow rate was set to 0.8 L/min, and Cool gas flow rate was maintained at 14 L/min.

Carminic acid method

The carminic acid method involved a modification of Hatcher and Wilcox's procedure to decrease the utilization of acid (Hatcher & Wilcox, 1950; Kuru et al., 2019). Boric acid (Merck, Darmstadt,

Fig. 1 Mineral water sources

Germany) was utilized in the preparation of boron standard solutions (0.5, 1, 2, 4, 8, and 10 mg/L) for the calibration curve. Concentrated HCl, concentrated H_2SO_4, and carmine solution were poured into the 0.2 mL mineral water sample as 10 µL, 1 mL, and 1 mL, respectively. After keeping the samples at room temperature for 45 min, absorbance readings were taken at a wavelength of 585 nm in a spectrophotometer (Rayleigh-UV-1800). All solutions were freshly prepared. The mean values of the five samples with three replicates from each brand were calculated.

Azomethine-H method

For the azomethine-H method (Koseoglu & Kolak, 2011) azomethine-H solution (0.25 mL) and ammonium acetate buffer solution (0.25 mL) were added to the mineral water sample (1 mL). Boric acid (Merck, Darmstadt, Germany) was utilized in the preparation of boron standard solutions (0.05, 0.1, 0.25, 0.5, 1, and 2 mg/L) for the calibration curve. After keeping the samples at room temperature for 1 h, absorbance readings were taken at a wavelength of 415 nm in a spectrophotometer (Rayleigh-UV-1800). All solutions were freshly prepared. The mean values of the five samples with three replicates from each brand were calculated.

Statistics

Pearson correlation analysis was conducted using IBM SPSS (15.0 version for Windows, Chicago, Illinois). The Bland Altman analysis was performed using Jamovi (version 2.3). Simple linear regression analysis was used to obtain the boron standard curve via GraphPad Prism 9.0.

Results and discussion

The mineral water boron levels obtained by three different methods are seen in Table 1.

The boron levels of mineral waters, determined by the ICP-MS technique, carminic acid,

Table 1 Boron concentration

	Boron levels (mg/L) ($n=105$) Mean ± SD (Range)
ICP-MS	3.08 ± 2.41 (0.09–8.61)
Carminic acid method	2.99 ± 2.20 (0.06–8.13)
Azomethine-H method	2.44 ± 1.92 (0.05–7.56)

ICP-MS inductively coupled plasma mass spectrometry, *SD* standard deviation, five bottle samples from each brand (total 21 brands) were included, therefore, total of 105 ($21 \times 5 = 105$) water samples were used to determine boron levels

and azomethine-H methods, differ between 0.09–8.61 mg/L, 0.06–8.13 mg/L, and 0.05–7.56 mg/mL, respectively (Table 1). The relative standard deviation (RSD) values ranged from 1.28 to 2.22% for ICP-MS, from 0.18 to 7.64% for carminic acid, and from 0.26 to 6.29% for the azomethine-H method. These were within the accepted variable limits < 10% RSD (USFDA 2015). The highest boron level has been found in Afyonkarahisar-sourced mineral waters and the lowest level has been observed in Giresun-sourced mineral waters (Fig. 2).

The level of boron in groundwater is usually less than 1 mg/L. Higher concentrations in natural waters may result from the dissolution of evaporites, hydrothermal action, seawater intrusion, residual seawater, evaporation concentration, mineral weathering, the process of sorption and desorption of B to mineral surfaces, and human pollution. The variations between boron levels observed in mineral waters could be linked to several factors. Distance of mineral water sources to boron mine can be one of them (Udagedar et al., 2014). The distance of mineral water sources to the nearest boron mine is shown in Fig. 3. Afyonkarahisar results validate the distance factor. Among the mineral waters we examined, mineral water from Afyonkarahisar, which is closest to a boron mine, showed the highest boron levels (Fig. 3). However, this trend was not consistent across all samples; for example, Karabük, despite being distant from boron mines, also exhibited high boron levels (Fig. 3). This indicates that distance may not be the sole determining factor for boron levels in mineral waters.

The solubility of boron compounds could also influence the levels of boron detected in the mineral waters (Luptáková & Derco, 2015). Tincal whose reserves are in Eskişehir-Kırka, has the highest solubility among boron compounds in Turkey (Turker, 2020). In support of this, the boron levels were at their peak in the mineral waters of Afyonkarahisar, which are the closest sources to Kırka (Fig. 3).

Precipitation may also be another factor. Cicek et al. observed that the concentrations of boron in the same regions vary seasonally, and they revealed that the reason can be precipitation (Cicek et al., 2012). Additionally, characteristics of rocks surrounding

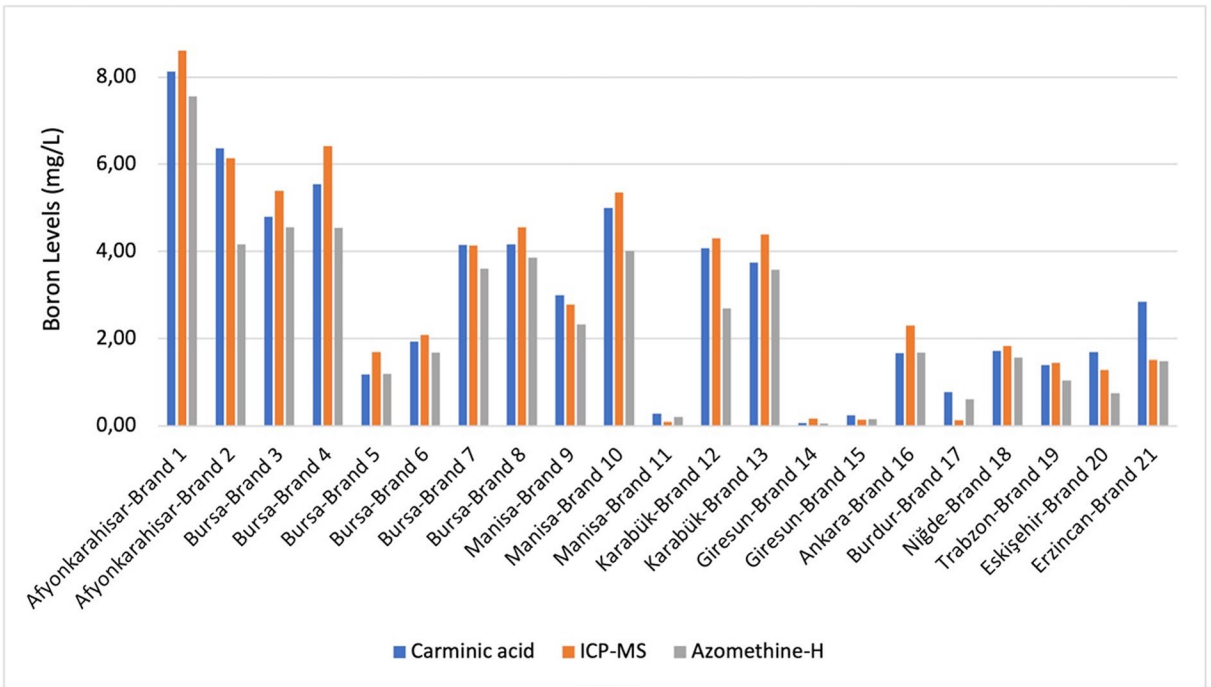
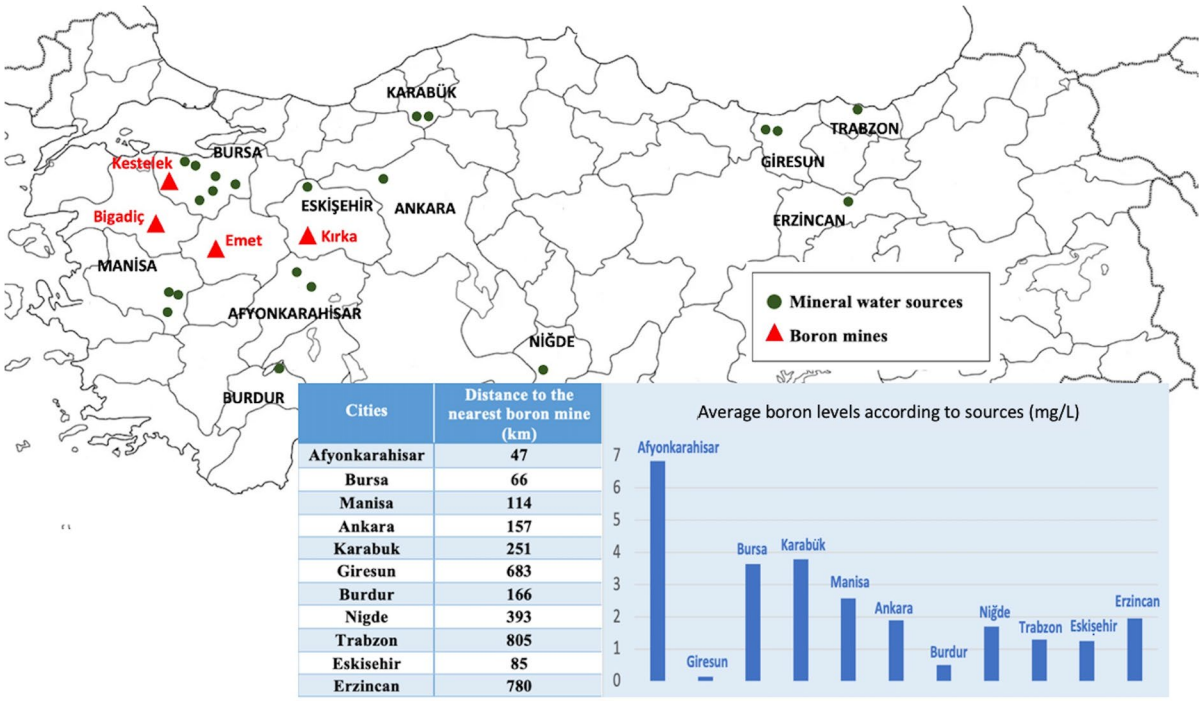


Fig. 2 Average boron levels of mineral waters analyzed in this study. The average boron concentration of five water samples was obtained from each brand, therefore, it is $n = 5$ for each brand



Cities	Distance to the nearest boron mine (km)
Afyonkarahisar	47
Bursa	66
Manisa	114
Ankara	157
Karabuk	251
Giresun	683
Burdur	166
Nigde	393
Trabzon	805
Eskisehir	85
Erzincan	780

Fig. 3 Distance between mineral water sources and boron mines

groundwater, age, and depth of groundwater may also affect the boron level of mineral waters (Davidson & Wilson, 2011).

While boron is necessary for human physiology, it is known that excessive amounts may lead to toxicity (Uluisik et al., 2018). As previously stated, WHO has established the tolerable upper intake level as 0.4 mg in 1 day per kg body weight (WHO, 1996).

Kuru et al. have evaluated boron levels in drinking water (Kuru et al., 2020) and foods (Kuru et al., 2019) in Turkey. The average amount of boron was determined as 0.2 mg/L for drinking water and 1.45 ± 0.4 mg/day for foods. When we consider the recommended water consumption is 2 L/day, approximately 0.4 mg of boron is taken from drinking water daily in Turkey. Thus, daily boron intake from diet is approximately 1.85 ± 0.4 mg/day in Turkey.

Our findings demonstrate that boron levels in mineral waters vary from a minimum of 0.05 mg/L to a maximum of 8.61 mg/L. Because one bottle is 200 mL, the boron content per bottle of mineral water ranges from a minimum of 0.01 mg/L to a maximum of 1.72 mg/L. When the daily upper limit level is taken into consideration, our research supports the safety of mineral water consumption in terms of boron levels. It helps fulfill our boron intake without surpassing the daily upper limit. Hence, it may help in the maintenance of homeostasis.

A strong correlation was observed between ICP-MS technique/carminic acid method results ($p < 0.0001$, Pearson correlation coefficient: $r = 0.948$), ICP-MS technique/azomethine-H method ($p < 0.0001$, Pearson correlation coefficient: $r = 0.898$), carminic acid method/azomethine-H method ($p < 0.0001$, Pearson correlation coefficient: $r = 0.876$) (Table 2).

Table 2 Correlation between boron determination methods

Correlations	r (Correlation coefficient)	P Pearson correlation
ICP-MS-Carminic acid	0.948	$p < 0.0001$
ICP-MS-Azomethine-H	0.898	$p < 0.0001$
Carminic acid-Azomethine-H	0.876	$p < 0.0001$

ICP-MS: inductively coupled plasma mass spectrometry, to correlate the three methods, all water samples were used for boron determination ($n = 105$)

Bland Altman analysis was conducted too (Fig. 4). For the ICP-MS technique/carminic acid method, the 95% limit of agreement is between 1.4 and 1.6, and a bias value was calculated as 0.1 in this graph. The *p*-value was found 0.2, which means there is no statistically significant difference between the two methods (Fig. 4a). On the other hand, *p*-values were found 0.0001 for both the ICP-MS technique/azomethine-H method (Fig. 4b) and carminic acid method/azomethine-H method (Fig. 4c). Therefore, a significant difference was observed between both the ICP-MS technique/azomethine-H method and the carminic acid method/azomethine-H method.

Intra-day and inter-day precision and accuracy for the modified carminic acid method and azomethine-H method were given in Tables 3 and 4. For the carminic acid method, the RSD values were found to be between 0.01 and 0.87% for intra-day precision and between 0.01 and 0.34% for the inter-day precision. The intra-day and the inter-day accuracy were between 99.3–115 and 98.9–118%, respectively. For azomethine-H method, the RSD values were found to be between 0.3 and 6.67% for intra-day precision and between 0.08 and 6% for the inter-day precision. The intra-day and the inter-day accuracy were between 99.0–120.0 and 99.5–110.0%, respectively.

RSD values for the modified carminic acid assay and the azomethine-H method were within the acceptable variability limits of less than 10% RSD (USFDA 2015). Intra-day and inter-day accuracies for both methods met the accepted variability range of 80–120% (Huber, 2010).

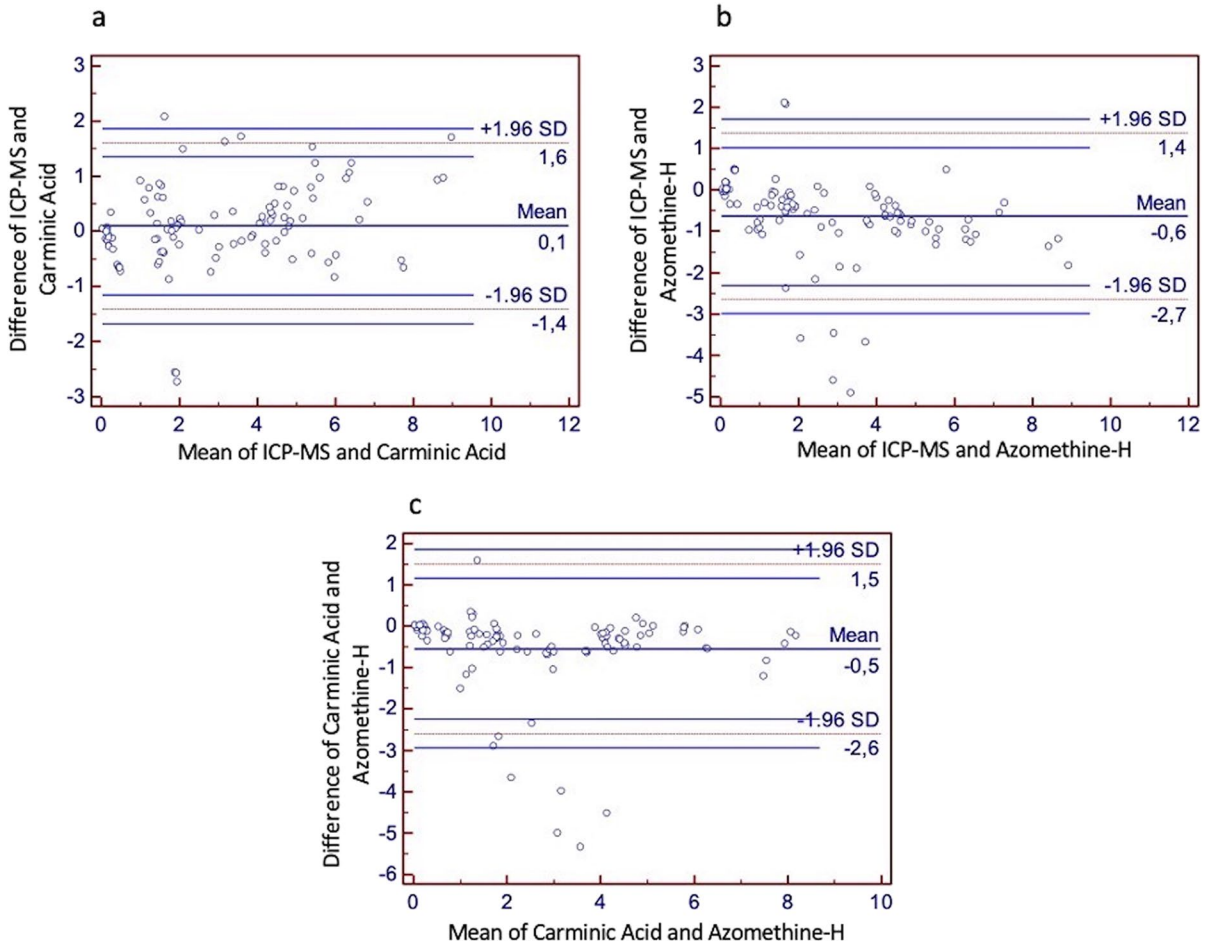


Fig. 4 The Bland Altman graph for **a** ICP-MS technique and carminic acid method, **b** ICP-MS technique and azomethine-H method, **c** carminic acid method and azomethine-H method. ICP-MS: inductively coupled plasma mass spectrometry

Table 3 Intra-day and inter-day precision and accuracy for modified carminic acid method

Intra-day $n=6$				Inter-day $n=6$			
Actual concentration (mg/L)	Found concentration (mg/L) ± SD	RSD %	Recovery %	Actual concentration (mg/L)	Found concentration (mg/L) ± SD	RSD %	Recovery %
0.5	0.53 ± 0.002	0.38	106.0	0.5	0.59 ± 0.002	0.34	118.0
1	1.15 ± 0.001	0.87	115.0	1	1.17 ± 0.002	0.17	117.0
2	2.17 ± 0.002	0.09	108.5	2	2.19 ± 0.002	0.09	109.5
4	4.09 ± 0.001	0.02	102.3	4	4.14 ± 0.005	0.12	103.5
8	7.94 ± 0.001	0.01	99.3	8	8.02 ± 0.006	0.07	100.2
10	9.95 ± 0.002	0.02	99.5	10	9.89 ± 0.001	0.01	98.9

RSD relative standard deviation

Table 4 Intra-day and inter-day precision and accuracy for azomethine-H method

Intra-day $n=6$				Inter-day $n=6$			
Actual concentration (mg/L)	Found concentration (mg/L) ± SD	RSD %	Recovery %	Actual concentration (mg/L)	Found concentration (mg/L) ± SD	RSD %	Recovery %
0.05	0.06 ± 0.004	6.67	120.0	0.05	0.05 ± 0.003	6.00	100.0
0.1	0.11 ± 0.005	4.55	110.0	0.1	0.11 ± 0.003	2.72	110.0
0.25	0.25 ± 0.001	0.40	100.0	0.25	0.26 ± 0.002	0.77	104.0
0.5	0.52 ± 0.002	0.38	104.0	0.5	0.51 ± 0.004	0.78	102.0
1	0.99 ± 0.014	1.41	99.0	1	1.01 ± 0.006	0.59	101.0
2	2.00 ± 0.006	0.30	100.0	2	1.99 ± 0.015	0.08	99.5

RSD relative standard deviation

In this study, the limit of detection (LOD) and limit of quantitation (LOQ) levels obtained as the output of the ICP-MS device were about 0.0003 ppm and 0.001 ppm, respectively (Fig. 5a). For carminic acid and azomethine assays' LOD and LOQ were calculated using 3.3 and 10 σ/s formulae, respectively, where, "σ" is the standard deviation of blank readings and "s" is the slope of the calibration curve (Shrivastava & Gupta, 2015). The LOD and LOQ of the carminic acid assay were 0.061 and 0.18 ppm (Fig. 5b) and for the azomethine-H method were 0.025 ppm and 0.074 ppm (Fig. 5c). The ICP-MS detection limit was very low compared to both two assays.

In the literature, several studies utilize carminic acid or azomethine methods for measuring boron concentrations (Türker & Türker, 2019). Discrepancies are observed in the LOD and LOQ values of these methods. These differences may stem from pretreatment procedures aiming to eliminate interferences, method modifications, or variations in sample preparation protocols.

The determination of boron content can be achieved through various methods, including colorimetric, fluorimetric, volumetric, potentiometric, atomic spectrometric, chromatographic, and ICP-MS techniques. Among these, colorimetric methods are commonly used, utilizing specific reagents to develop color for boron determination. Popular examples include curcumin, carmine, arsenazo, methylene blue, azomethine H, and crystal violet methods. The most frequently used methods in the literature are colorimetric and ICP-MS methods. In this study, boron analysis in mineral waters was conducted using ICP-MS, carminic acid, and azomethine-H methods, each offering unique strengths and limitations. The selection of a method depends on factors such as sensitivity requirements, sample matrix complexity, cost considerations, and equipment availability.

ICP-MS is a technique used to measure the concentration of elements in a sample by ionizing the atoms in an argon plasma and analyzing the mass-to-charge ratios of the ions produced. While it offers

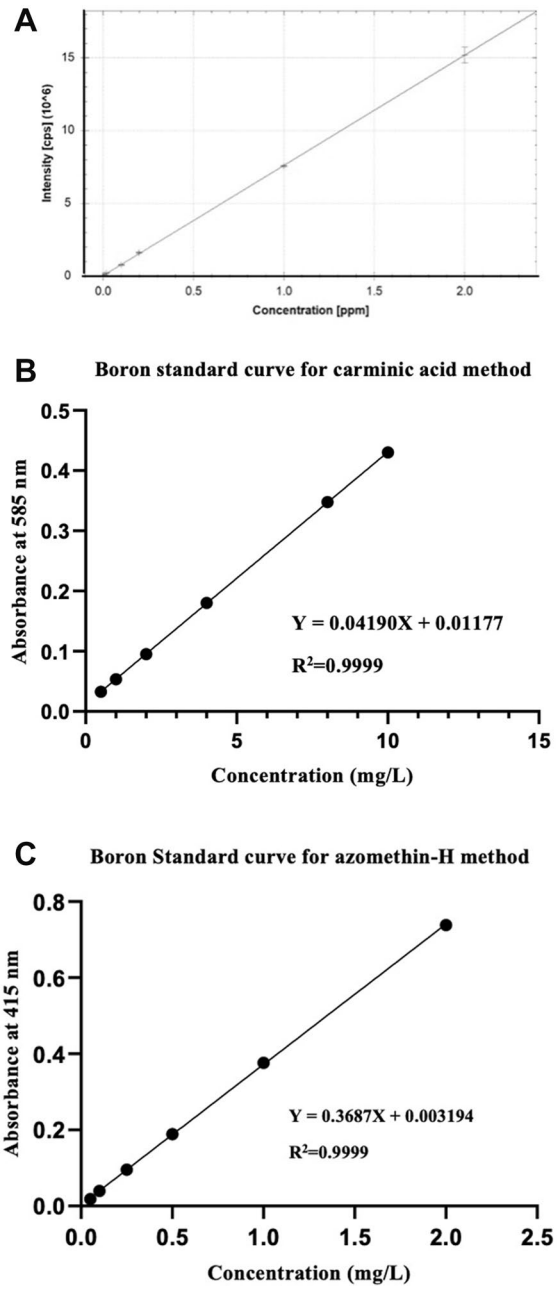


Fig. 5 Boron calibration curves for three methods. **A** For ICP-MS method (Calibration equation $Y = 7579668.4591X + 16202.6848$; $R^2 = 1.0000$; LOD = 0.0003 ppm; LOQ = 0.001 mg/L; **B** for carminic acid method (Calibration equation $Y = 0.04190X + 0.01177$; $R^2 = 0.9999$; LOD = 0.061 ppm; LOQ = 0.18 mg/L; **C** for azomethine H method (Calibration equation $Y = 0.3687X + 0.003194$; $R^2 = 0.9999$; LOD = 0.025 ppm; LOQ = 0.074 mg/L

high sensitivity and is, ICP-MS requires expensive equipment and expertise (Simsek et al., 2003). As alternatives, the carminic acid and azomethine-H methods offer simpler and more affordable options for routine boron analysis in water samples with moderate sensitivity requirements (Sah & Brown, 1997). These methods, while less sensitive compared to ICP-MS, are suitable for routine analysis and may have fewer limitations in terms of cost and complexity.

ICP-MS typically has higher interference tolerance because of its selectivity and ability to separate ions based on mass-to-charge ratios. However, sample preparation and matrix effects can still affect measurement accuracy. Carminic Acid and Azomethine H methods may encounter interferences from other substances, requiring careful optimization and validation to ensure accurate boron determination in water samples (Sah & Brown, 1997). The advantage of the carminic acid method is not interfere with various substances commonly found in water and plant materials (Hatcher & Wilcox, 1950). Compared to other colorimetric methods, it provides accurate and reproducible results for boron determination in water, soil, and plants (Callicoat & Wolszon, 1959; Floquet, et al., 2016). However, it requires fresh preparation of boron standard and carmine solutions. While the method traditionally involves the use of concentrated sulfuric acid, modified versions with reduced sulfuric acid usage have been developed to address this concern (Kuru et al., 2019). The Azomethine-H method for boron determination in water samples may face various interferences such as transition metal ions forming colored complexes, anions causing spectral overlap, pH variations influencing color intensity, organic compounds interfering with reaction kinetics, and oxidizing agents disrupting color development (Table 5). To mitigate these interferences, precise control of experimental conditions, proper sample preparation, selective masking agents when necessary, calibration with standards, and rigorous quality control measures are essential (Sah & Brown, 1997). The choice of method depends on the specific requirements of the analysis, budget constraints, and available resources. As a masking agent, EDTA is used for the azomethine-H method, and HCl is used for the carminic acid method to increase sensitivity.

Our findings show that the ICP-MS technique exhibits lower LOD values compared to both methods. However, since boron concentrations in mineral

Table 5 Possible interferences of carminic acid, azomethine-H methods, and ICP-MS technique

Method	Interferences	Masking agents	Reference
Carminic acid	Metal ions, NO_3^-, NO_2^-	HCl	Floquet et al., 2016; Sahin, 2007; Sah & Brown, 1997; Rosenfeld & Selmer-Olsen, 1979; Hatcher et al., 1950
Azomethine-H	Fe, Cu, Al, Zn, HCO_3^-, F^-, K	EDTA, DTPA, EDTATioglycolic acid, EDTA-mannitol, methyl borate	Floquet et al., 2016; Sahin, 2007; Carrero et al., 2005; Sah & Brown, 1997; Spencer & Erdmann, 1979
ICP-MS	Ionic strength matrices	Dilution	Floquet et al., 2016

waters are sufficiently high, they are within the measurement limits of the azomethine and carminic acid methods. Thus, the amount of boron can also be determined using these spectrophotometric methods.

Additionally, results obtained from the carminic acid method showed a strong correlation with those from the ICP-MS technique. Most of the differences were between the agreement limits and no significant difference was observed between the two methods. Therefore, it can be said that there is agreement in the measurements between the two methods, indicating that the carminic acid method seems suitable for boron analysis when the ICP-MS technique is unavailable.

On the other hand, a significant difference was found between the azomethine-H method and both the ICP-MS technique and the carminic acid method. The azomethine-H method is a frequently used spectrophotometric method for the determination of boron level because it is cheap and sensitive. Various studies achieved good results using it (Sarkar et al., 2014; Simsek et al., 2003). However, the main drawback is its high cross-sensitivity with other elements (Carrero et al., 2005; Floquet et al., 2016). Since natural mineral waters contain many elements in their nature, this may cause serious interference, affect the results, and cause significant differences between azomethine-H and other methods.

Conclusion

The mineral waters sourced in Afyonkarahisar have shown the highest boron content, whereas those from Giresun have demonstrated the lowest boron levels. Unless the daily consumption exceeds about 14 bottles/day, drinking mineral water in Turkey cannot exceed the daily upper limit defined by the WHO. As boron plays a beneficial role in human physiology, natural mineral water has the potential to benefit public health by supporting boron intake. According to our outcomes, the spectrophotometric carminic acid method can yield results similar to those obtained using the ICP-MS technique. However, it should be noted that the boron levels of the analyzed mineral waters were within the detection limits of the carminic acid method. If they were not, we would have to use ICP-MS. Therefore, the carminic acid method is suitable for determining boron levels within the detection limits in the absence of the ICP-MS technique.

Author contributions Armagan Begum Ozel Korlu: methodology, investigation, resources, analysis, writing original draft, writing review, editing, and visualization. Sahin Yilmaz: investigation, resources, analysis. Ozlem Sacan: conceptualization, methodology, resources, editing, and supervision. Refiye Yanardag: conceptualization, methodology, resources, editing, and supervision. Aysen Yarat: conceptualization, methodology, formal analysis, resources, writing original draft, writing review, editing, and supervision. Fikrettin Sahin: conceptualization, methodology, resources, editing, and supervision.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests The authors declare no competing interests.

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