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Method Validation for The Determination of Toxic Elements in Fizzy Fruity Mineral Water Drinks Using ICP-MS

Murat Kilic[1], Serpil Kilic[1*]

*[*1] Akdeniz University, Food Safety and Agricultural Research Center, Antalya, Turkey*

*Corresponding author :serpilkilic@akdeniz.edu.tr
Orcid No: http://orcid.org/0000-0002-4940-1839

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Abstract: The presence of metallic impurities in fizzy fruity mineral water drinks can constitute health hazards to the public. In this study, the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was chosen to be validated, and applied in suitable method of analysis for determination of antimony, lead and cadmium in samples. The detection limits, quantification limits, linearity, accuracy parameters were studied under optimised ICP-MS conditions. The method trueness was confirmed by using certified references materials LGC soft drink and obtained results had acceptable Z-score values. The results obtained make the validated method suitable for a precise determination of the toxic elements in different brands of samples at these low concentration values. The results obtained were checked with permissible levels, daily intake (EDI), target hazard quotient (THQ) and hazard index (HI).

Keywords: Fizzy fruity mineral water, Health risk, ICP-MS.

1. Introduction

Fizzy fruity mineral water drinks are non-alcoholic beverages that typically contain carbonated water. Although regular intake of carbonated soft drinks has been associated with human health problems, carbonated mineral waters remain one of the most frequently consumed ready to drink beverages in the world (Barbara et al., 2015). The high consumption rate of fizzy drink is attributed to the characteristic taste and flavour as well as their thirst quenching potential (Phillip et al., 2013).

The presence of some heavy metals in these drinks which may be due to environmental pollution from surface and underground water have been reported (Galadima et al. 2012). Lead, cadmium and antimony are non-essential metals as they are toxic even in trace amounts. Toxic elements can be very harmful, even at low concentration. According to the United States Environmental Protection Agency (USEPA), and the International Agency for Research on Cancer (IARC), these metals are also classified human carcinogens based on epidemiological studies (IARC, 2006). Human exposure to these toxic metals has risen as a result of increase of their uses in several industrial, agricultural and technological applications (Tchounwou et al., 2012). Trace elements are very important due to their adverse health effects on human metabolism, and therefore, their analysis methods are also an important part of public health studies. The monitoring of heavy metals in water and foods are therefore of great importance in protecting the public from the hazards related to these metals (Lee et al., 2006; Mendil et al., 2010; Kilic et al., 2015; Kilic et al., 2018a; Kilic et al., 2018b).

Therefore, this study presents the establishment and validation of analytical method for determination of Pb, Cd and Sb in fizzy fruity mineral water drinks using ICP-MS. The work described here establishes a simple and efficient method for sample preparations including additional different kinds of soft drink samples. Results obtained were compared with permissible levels set out by WHO (World Health Organization) and evaluated for the risks to human health. The analytical performances of the method such as linearity, limit of detection (LOD), limit of quantification (LOQ), specificity/selectivity and recovery (%) were determined. The obtained results were checked with permissible levels, daily intake (EDI), target hazard quotient (THQ) and hazard index (HI).

2. Materials and Method

2.1. Reagents

Stock calibration standard solutions VHG (10 mg/L) of Pb, Cd and Sb prepared in 1% HNO_3 were purchased from Manchester, USA. Suprapur® concentrated nitric acid (HNO_3) 65 % (w/w) was purchased from Merck-Germany. Deionized water was produced using Water Purification

System equipped with Millipore® ultrapure (Bedford, USA).

2.2. Standards preparation

Standard solutions were prepared by diluting 10 mg/L of metals stock standard solutions of Pb, Cd, Sb to 100 mL with 2 % (v/v) HNO_3. Six working standard solutions, covering a range of 0.5-50 µg/L were prepared by diluting intermediate standard solutions with 2 % (v/v) HNO_3.

2.3. Sample preparation

18 fizzy fruity mineral water drinks samples were collected for this study and were carried to the laboratory in the original glass bottles. Bottle caps of samples were opened at the moment of sample preparations and submitted to degassing process in ultrasonic bath for 15 min. After this step, samples were diluted to 50 mL by using 2% (v/v) HNO_3 solution and the samples were analysed by using ICP-MS. The optimized conditions and values of ICP-MS are summarized in Table 1.

Table 1. ICP-MS operating conditions

Spectrometer	Elan DRC-e (Perkin Elmer SCIEX, Norwalk, CT, USA)
Sample Introduction	Scott Spray Chamber
RF Power	1000
Skimmer Cone	Nickel
Sampler Cone	Nickel
Gas flow rates (L min^{-1})	Nebulizer gas flow: 0.91, Auxillary gas flow:1.20 Plasma gas flow:17
RF Power	1000
Lens Voltage	6.50
Nebulizer	Meinhard TQ plus Quartz 0.5 ml
Scannig mode	Peak hopping
Analytical masses (amu)	Standart mode ^{121}Sb, ^{208}Pb, ^{111}Cd
Number of sweeps/reading	20
Number of readings /replicate	1
Number of replicates	3
Auto sampler	CETAX ASX-520
Dwell time per AMU (ms)	50
Sample flush	Time (50), speed (+/- rpm)-48
Read delay	Time (15), speed (+/- rpm)-20

2.4. Validation parameters

Several parameters have been taken into account and evaluated for the method validation, namely, linear range, method linearity, recovery at three levels (minimum, medium and maximum) LOD, LOQ, trueness by CRM, and method repeatability. Analytical method validation of ICP-MS for the determination of Pb, Cd, Sb was applied in accordance with Eurachem Guide, LGC-soft drink BV 223 Round 519 reference material standart (EURACHEM, 1998).

2.5. Health risk assessment

The estimated daily intake (EDI) of toxic metal in this study was determined by the Eq. (1), as reported by WHO, (2009).

$$Daily\ intake\ (EDI) = \sum\left[\frac{Concentration\ of\ toxic\ metals\ in\ food\ xMean\ food\ intake}{Body\ weight}\right] \quad (1)$$

Dietary intake of toxic metals determined in this study were compared with the provisional tolerable weekly intake (PTWI) by JECFA (UNEP/FAO/WHO, 1992). Target Hazard Quotient is described by the following Eq. (2) by USEPA (USEPA, 2009).

$$THQ = \frac{(EFxEDxFIxMc)}{RfDxBWxAT} x10^{-3} \quad (2)$$

where THQ = target hazard quotient, EF = exposure frequency, ED = exposure duration equivalent to average life time, FI = food mean ingestion rate, MC = element concentration in the samples, AT = average exposure of life time and BW is average body weight. Reference oral doses (RfD) used for Cd, Pb and Sb were 1 x 10^{-3}, 4 x 10^{-3} and 4 x 10^{-4} mg/kg/day, respectively (USEPA, 2013).

The hazard index (HI) is the sum of the individual target hazard quotients of the elements assessed. The health risks associated were evaluated according to Eq. (3).

$$HI = Total\ THQ = THQ\ (As) + THQ\ (Cd) + THQ\ (Pb) \quad (3)$$

3. Results and Discussion

The assay analytical method developed was subjected to validation by performing specificity, linearity, limits of detection and quantification, precision and accuracy. The accepted values of correlation coefficients were greater than 0.995, as set, as target to obtain accurate quantification as an analytical linear response over certain concentration ranges. The LOD for the elements investigated were found to be in the range of 0.03 and 0.14 µg L^{-1}. The limits of quantification, expressed as the lowest validated spike level with acceptable criteria for accuracy and precision. The minimum practical concentrations of tested elements in the analyzed drinks, which can be determined with acceptable accuracy and precision were performed by analyzing ten replicates (1 µg L^{-1}) for Sb, Cd, and Pb. In order to verify the recovery, standard reference materials from LGC soft drink was analyzed using the validated method. The relative standard deviation (RSD) was ranged between 1.0 and 4.8 % (Table 2).

Table 2. Validation parameter results

Elements	Regression equation	Linear range (µg L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})	% RSD
Pb	y = 14524x – 2116	0.5-50	0.14	0.45	4.8
Cd	y = 2541.4x + 86.385	0.5-50	0.06	0.20	2.0
Sb	y = 5796.4x – 591.97	0.5-50	0.03	0.11	1.0

For precision calculations, spiking was performed at three fortification levels. The solution was spiked with minumum (1 µg L^{-1}), medium (15 µg L^{-1}), and maximum (40 µg L^{-1})levels and analyzed by the proposed method for Sb, Pb, and Cd. The recoveries were found between 95 and 101% for 1 µg L^{-1}, 97 and 105% for 15 µg L^{-1}, and 99 and 103% for 40 µg L^{-1} concentrations. Results are submitted in Table 3.

Table 3. Recovery values (%) of the elements

Elements	Recovery values		
	Minimum (1 µg L^{-1})	Medium (15µg L^{-1})	Maximum (40 µg L^{-1})
Pb	95±4.5	105±1.3	99±1.9
Cd	99±2.0	97±2.7	103±0.9
Sb	101±1.1	100±2.6	99±2.1

The Z-score is a statistical measure that quantifies the distance (measured in standard deviations) a data point is from the mean of a data set. All measured results were within satisfactory range and had acceptable Z-score ($-2 \leq$ Z-score ≤ 2).

Table 4.. Element composition of samples (µg L^{-1})

Sample		Sb	Pb	Cd
Fizzy fruity mineral water (n=18)	Min	<LOD	<LOD	<LOD
	Max	<LOD	0.5	<LOD
	Mean	<LOD	0.2	<LOD
	Std. Deviation	<LOD	0.2	<LOD
WHO 2011		20	10	3
EU 2014		5	10	5

Eighteen natural fizzy fruity mineral water having different brands and most consumed were analyzed by the developed method. According to the results in **Table 4.** cadmium and antimony could not be determined (<LOD), in the samples. Lead was determined in the some brand samples. As reported in a study completed in Poland, 0.14 µg L^{-1} lead, 1.32 µg L^{-1} arsenic and 0.45 µg L^{-1} cadmium have been detected in bottled mineral waters samples purchased from markets (Astel et al., 2014). The results obtained for the lead and arsenic contents were very low compared to literature data. All these results were found to be much lower than the maximum permissible limits of metals contaminants stated by WHO, and Europeans standards in drinking and potable bottled natural mineral water (EU, 2003; WHO, 2006).

Dietary intake calculations were made according to the Turkey Development Bank data reports. Development Bank of Turkey reported an average body weight for Turkish population as 66.5 kg, while natural mineral water consumption for population is 4.4 L/year. Compared to the safety standard set by FAO/WHO Expert Committee on Food Additives (JECFA) for antimony, a PTWI of 6 µg kg^{-1} bw; for lead, a PTWI of 25 µg kg^{-1} bw; for cadmium, a PTWI of 7 µg kg^{-1} bw (FAO/WHO, 1997; WHO, 2003).The estimated lead intake level from this study correspond lower from the PTWI.

The THQ results was determined $9x10^{-6}$ for Pb. The parameters used for risk assessment give an estimation of the human risk evaluation of exposure to metal in the samples stated. THQ value less than 1 indicates that there is no significant risk of noncarcinogenic effects for the exposure (USEPA, 1989). The results showed that the consumption rate of fizzy fruity mineral water drinks in Turkey can not result over exposures to these chemicals.

4. Conclusions

In this study, ICP-MS technique was used to validate an analytical method for the determination of toxic-trace elements in fizzy fruity mineral water drinks. The validated method proved to be fast, easy and simple, and therefore, it can be very useful for routine laboratory applications. Precise and accurate results were obtained with the validated method even at very low levels of concentrations. According to the results of the natural mineral water analyses, Cd concentration levels were at the LOD levels. Arsenic and lead were detected in some samples. The method quantification limits were found to be much lower than the maximum permissible limits of metal contaminants set by WHO and Europeans Standards in drinking and bottled natural mineral waters. The health risk assessment indices generally indicated no risk concern with respect to human exposure and public health. Therefore, this might be considered as a useful tool by governmental organizations.

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Authors' contributions: S. Kilic designed the study, interpreted the heavy metal results and M. Kilic collected samples from different locations, analyzed for heavy metals.

Conflict of interest disclosure: The authors declare that they have no conflict of interest.

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