



Article

A Comparative Analysis of the Oral Bioaccessibility of Metals/Metalloids Determined Using the Unified Bioaccessibility Research Group of Europe Method and 0.07 M HCl Single Extraction Method

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Abstract: Metals and metalloids in consumer products can be ingested by humans and cause health problems. The unified Bioaccessibility Research Group of Europe (BARGE) method (UBM, i.e., ISO 17924), with complex digestive ingredients, and the 0.07 M HCl single extraction method, as a simplified means, have been widely used to assess oral bioaccessibility in vitro. Herein, the bioaccessible concentrations of metals and metalloids in 13 certified reference materials acting as surrogates for consumer products were determined using the UBM and the 0.07 M HCl single extraction method and compared. The bioaccessible concentrations of metals and metalloids evaluated using the UBM and the 0.07 M HCl single extraction method ranged from 0.002 to 17,449 mg/kg and from 0.003 to 20,283 mg/kg, respectively; their bioaccessibility ranged from 0.00002 to 26.9% and from 0.00002 to 36.6%, respectively. The 0.07 M HCl single extraction method showed higher concentrations, as the bioaccessible concentrations of metals and metalloids differed by 1.38 times (i.e., the slope of the linear regression), indicating its potential for conservative assessment. However, the Student's *t*-test results for the 12 metals and metalloids showed no significant differences (p -value ≥ 0.05). It demonstrated that the relatively simple 0.07 M HCl single extraction method can be used as an in vitro test method to assess the oral bioaccessibility of metals and metalloids in various consumer products by replacing the UBM and/or through its use as a screening method prior to the application of the UBM, thereby moving towards green analytical chemistry.

Keywords: oral ingestion; migration; risk assessment; single extraction; green analytical chemistry

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1. Introduction

Metals contained in various consumer products, including toys, cosmetics, glass bottles, and chopsticks [1–4], can be introduced into the human body through oral ingestion. Guney et al. [1] investigated the concentrations of arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb), and selenium (Se) in 72 children's products and observed that 32 of them showed levels that exceeded the metal standards suggested by the EU Directive 2009/48/EC [2]. Additionally, Cui et al. [3] reported that according to the concentrations of As, Cd, Cr, Ni, Pb, and Sb in children's products, 16 out of 45 products exceeded the guidelines. Along with the possibility of oral exposure to products due to children's sucking behavior, adults have also used several products with oral exposure concerns, and the detection of metals in those products has also been reported. The concentrations of Cd and Pb in the enamel of a glass bottle tested by Turner [4] were 100,000 mg/kg, and 20,000 mg/kg, respectively. Similarly, Zhao et al. [5] reported that the concentrations of Cd, Co, Cr, Ni, and Pb in paint on

chopsticks distributed to consumers were up to 120,000 mg/kg, 2600 mg/kg, 8500 mg/kg, 150,000 mg/kg, and 500,000 mg/kg, respectively. Therefore, careful assessments of the migration of metals from various consumer products into the human body through oral exposure routes should be thoroughly performed to ensure human health.

Oral bioaccessibility indicates the fraction that can be absorbed by the human body upon the ingestion of metals contained in confined matrices and can be used as an indicator of reliable human health risk, being better than total metal concentrations. The amount of metal actually absorbed through the gastrointestinal tract can be applied for human health risk assessment, considering relative bioavailability (RBA), through *in vivo* assays but faces many economic and ethical limitations [6,7]. In this regard, *in vitro* test methods have been proposed to determine the bioaccessibility of metals in confined matrices, with a high correlation with *in vivo* assay results. The various test methods with a good *in vitro*–*in vivo* correlation (IVIVC), which were suitable for assessing the oral bioaccessibility of metal-contaminated soil, include the *in vitro* gastrointestinal method (IVG) [8], the physiologically based extraction test method (PBET) [9], OSWER 9285.7-77 of the United States Environmental Protection Agency (USEPA) [10], ISO 17924 of the International Standards Organization (ISO) [11], and DIN 19738:2 of the Deutsches Institut für Normung e.V. (DIN) [12]. One can assess the oral bioaccessibility of children's products through BS EN 71-3 of the British Standards Institution (BSI) [13], simulating gastric juice with 0.07 M HCl; EUR 19899 EN of the Joint Research Centre European Commission (JRC) [14], simulating saliva with MgCl₂, CaCl₂, K₂HPO₄, K₂CO₃, NaCl, and KCl; and Report 320102004 of the National Institute for Public Health and the Environment (RIVM) [15], using human body fluids containing 12 inorganics, 4 organics, and 8 enzyme components. Among them, ISO 17924, also known as the unified Bioaccessibility Research Group of Europe (BARGE) method (UBM), is one of the most detailed simulations of human digestive juices and their processes.

Armenta et al. [16] proposed the use of green analytical chemistry to reduce energy and reagent consumption, waste generation, and the time required while also simplifying the test process. In this respect, the *in vitro* bioaccessibility tests with artificial digestive juices containing complex components faced a situation that required in-depth research on the possibility of their replacement with a more simplified testing method. For this purpose, Pelfrère et al. [17] compared bioaccessible metal concentrations extracted using 0.65% HCl (0.21 M HCl), acetic acid, ethylenediaminetetraacetic acid (EDTA), citric acid, and artificial digestive juices (according to the UBM) in 201 soil samples. A positive correlation between the results obtained using the UBM and the 0.65% HCl extraction method was found, with a coefficient of determination $R^2 = 0.91$ for As, $R^2 = 0.97$ for Cd, and $R^2 = 0.72$ for Pb [18]. These results indicated that 0.65% HCl could be suitable for a single extract from the soil to assess the bioaccessibility of metals instead of using the complex artificial digestive juices. Other studies also proved that the UBM could be replaced with 0.43 M HNO₃ for As, Ba, Cd, Cu, Ni, Pb, and zinc (Zn) in soil [18,19]. Comparative experiments on environmental media such as soil have been performed, but none on consumer products such as children's toys have been performed.

In this study, the oral bioaccessibility of 12 metals and metalloids (As, Ba, Cd, Cr, Cu, Ni, Pb, Sb, Se, tin (Sn), strontium (Sr), and Zn) was assessed using the UBM as a precise means to mimic the human digestive processes and the 0.07 M HCl single extraction method as a simplified means. EN 71-3 (i.e., 0.07 M HCl single extraction method) was selected for comparison as it has many applications for children's products, uses fewer reagents, produces less waste, and has a simpler test method. Currently, the National Institute of Environmental Research (NIER) in Korea monitors bioaccessible metal concentrations in children's products using the 0.07 M HCl single extraction method, presented in BS EN 71-3 of the BSI [13]. The correlation between the test results obtained using the two methods was examined, and the possibility of replacing the UBM with the single extraction method was investigated.

2. Materials and Methods

2.1. Certified Reference Materials

For a quantitative comparison between the oral bioaccessibility assessment methods, certified reference materials (CRMs) were used as homogeneous samples with known concentrations of various elements rather than actual consumer products, which may involve sampling errors owing to their heterogeneity. A total of 13 types of CRMs were used, which had different materials, metal/metalloid types, and metal/metalloid contents. The selected metal/metalloid types were As, Ba, Cd, Cr, Cu, Ni, Pb, Sb, Se, Sn, Sr, and Zn, which are regulated according to the EU Directive 2009/48/EC related to the safety of toys [2].

As plastic CRMs, we used CRM 113-01-013 (acrylonitrile butadiene styrene (ABS), Korea Research Institute of Standards and Science, Daejeon, Republic of Korea); JSM P 701-1 (polyethylene (PE), JFE Techno-Research Corporation (JFE-TEC), Tokyo, Japan); ERM-EC680m (PE) and ERM-EC681m (PE) (European Reference Materials (JRC Geel), Geel, Belgium); SRM 2861 (polyvinyl chloride (PVC), National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA); and JSAC 0602-3 (polyethylene terephthalate (PET), Japan society for analytical chemistry (JSAC), Tokyo, Japan). As CRMs of paint materials, 110-05-paint-02 (Korea Testing and Research Institute (KTR), Gyeonggi-do, Republic of Korea) and CRM 013-50G (Sigma-Aldrich, Saint Louis, MO, USA) were used. As CRMs of glass materials, BAM-S004 (Federal Institute for Materials Research and Testing (BAM), Göttingen, Germany) was used. Finally, as metal CRMs, SRM 856a (aluminum; NIST), SRM 875 (cupro-nickel; NIST), SRM 899 (nickel-base; NIST), SRM 872 (phosphor bronze; NIST), and 102-02-SBSI5 (silicon; KTR, Republic of Korea) were used. Table 1 lists the details of the metal/metalloid types and contents of the CRMs used (Table 1).

2.2. Determination of In Vitro Oral Bioaccessibility

2.2.1. Procedures of the UBM

Simulated digestive fluids are mainly classified as saliva, gastric fluid, duodenal fluid, and bile. An inorganic solution (250 mL) and organic solution (250 mL) were prepared by adding each constituent (Table 2), agitated for a sufficient time, and mixed into a 500 mL solution. The pH was adjusted to 6.5 ± 1.1 for the simulated saliva, 1.1 ± 0.1 for the simulated gastric fluid, 7.4 ± 0.2 for the simulated duodenal fluid, and 8.0 ± 0.2 for the simulated bile using 37% HCl and 1 M NaOH. Then, solid enzyme components (Table 2) were added to the 500 mL solution and agitated for a sufficient time.

The simulated saliva (9 mL) and gastric fluid (13.5 mL) were added to 0.6 g of the CRM. The sample was stirred for 1 h at 55 rpm in a constant-temperature water bath at 37 °C. Simulated duodenal fluid (27 mL) and simulated bile (9 mL) were then added to the sample. The sample was stirred for 4 h under the same agitation conditions. The sample was then subjected to 5 min of centrifugation at $4500 \times g$ for solid–liquid separation. After separating the supernatant from the sample, it was acidified by adding 1 mL of 67% HNO₃ and filtered through a 0.45 µm filter. The filtrate was analyzed using an inductively coupled plasma mass spectrometer (ICP-MS, 7900, Agilent Technologies, Santa Clara, CA, USA) (Figure 1a).

Table 1. Certified metal concentrations in the 13 CRMs used in this study.

CRM	Material ^a	Total Metal Concentration (mg/kg)											
		As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Se	Sn	Sr	Zn
JSM P 701-1	PE	187 ± 11.7	- ^b	114 ± 2.4	115 ± 3.8	-	-	111 ± 7.5	-	-	-	-	-
CRM 113-01-013	ABS	132 ± 4.7 ^a	-	14 ± 0.38	141 ± 3.2	-	147 ± 9.3	136 ± 3.6	152 ± 12	-	-	-	-
JSAC 0602-3	PET	-	-	50 ± 05	113 ± 1.8	-	-	112 ± 1.4	-	-	-	-	-
ERM EC680m	PE	4.7 ± 0.4	-	21 ± 0.9	9.6 ± 0.5	-	-	11 ± 0.4	9.6 ± 0.7	-	21 ± 1.6	-	194 ± 12
ERM EC681m	PE	17 ± 1.2	-	146 ± 5	45 ± 1.9	-	-	70 ± 2.5	86 ± 7	-	99 ± 6	-	1170 ± 40
SRM 2861	PVC	239 ± 5.1	740 ± 13	65 ± 3.6	-	50 ± 3.1	-	88 ± 4.7	68 ± 4	244 ± 3.2	1294 ± 10	-	-
BAM S004	Glass	-	-	-	471 ± 25	-	-	-	-	-	-	-	-
110-05-paint-02	Paint	-	-	226 ± 4	-	-	-	944 ± 6	-	-	-	-	-
CRM 013-50G	Paint	-	-	38 ± 1.21	618 ± 12	-	-	643 ± 13	-	-	-	-	-
SRM 856a	Aluminum	-	-	-	600 ± 10	35,000 ± 400	3700 ± 100	1100 ± 100	-	-	1000 ± 100	180 ± 20	9600 ± 100
SRM 872	Phosphor bronze	-	-	-	-	873,600 ± 200	-	41,300 ± 300	-	-	41,600 ± 500	-	40,000 ± 100
SRM 875	Cupronickel	-	-	22 ± 5	-	878,300 ± 500	104,200 ± 300	92 ± 5	<10	4	90 ± 30	-	1100 ± 100
SRM 899	Nickel alloy	-	-	-	-	-	-	39,280 ± 890	-	93,600 ± 2400	-	-	-

^a PE, polyethylene; ABS, acrylonitrile butadiene styrene; PET, polyethylene terephthalate; PVC, polyvinyl chloride. ^b -: Elements not contained in the CRM.

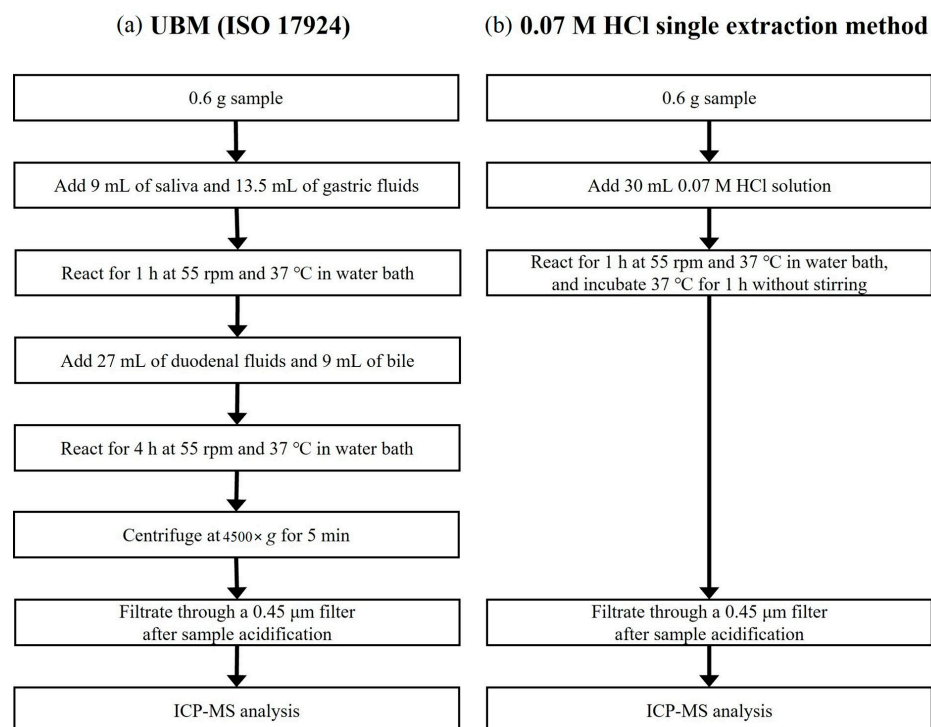


Figure 1. Procedures of (a) the UBM and (b) the 0.07 M HCl single extraction method to assess the oral bioaccessibility of metals.

Table 2. Composition of digestive fluids (inorganic solution 250 mL + organic solution 250 mL) with enzymes used in the UBM (ISO 17924 [11]).

Digestive Fluid Ingredients		Saliva	Gastric	Duodenal	Bile
Inorganic constituents (250 mL)	KCl	448 mg	412 mg	282 mg	188 mg
	NaH ₂ PO ₄	444 mg	133 mg		
	KSCN	100 mg			
	Na ₂ SO ₄	285 mg			
	NaCl	149 mg	1376 mg	3506 mg	2630 mg
	CaCl ₂		200 mg		
	NH ₄ Cl		153 mg		
	NaHCO ₃			2803.5 mg	2893 mg
	KH ₂ PO ₄			40 mg	
	MgCl ₂			25 mg	
NaOH (1 M)	0.9 mL				
HCl (37%)		4.15 mL	90 μL	90 μL	
Organic constituents (250 mL)	urea	100 mg	42.5 mg	50 mg	125 mg
	glucose		325 mg		
	glucuronic acid		10 mg		
	glucosamine hydrochloride		165 mg		
Enzymes ^a	α-amylase	72.5 mg			
	mucin	25 mg	1500 mg		
	uric acid	7.5 mg			
	bovine serum albumin		500 mg	500 mg	900 mg
	pepsin		500 mg		
	CaCl ₂			100 mg	111 mg
	pancreatin			1500 mg	
	lipase			250 mg	
	bile salts (bovine)				3000 mg
pH ^b		6.5 ± 0.5	1.1 ± 0.1	7.4 ± 0.2	8.0 ± 0.2

^a Solid enzymes were added to the mixture solution (500 mL). ^b Values after mixing the inorganic and organic solutions.

2.2.2. Procedures of the 0.07 M HCl Single Extraction Method

According to the NIER, the oral bioaccessibility of metals contained in children's products can be determined using 0.07 ± 0.005 M HCl [20]. A total of 30 mL of 0.07 M HCl solution was added to 0.6 g of the CRM. The sample was stirred for 1 h at 55 rpm in a constant-temperature water bath at 37 °C and incubated for 1 h without stirring at 37 °C. The sample was then filtered through a 0.45 µm filter to recover the filtrate for ICP-MS analysis (Figure 1b).

2.3. Metal/Metalloid Analysis and Data Treatment

The metal concentration was analyzed using an ICP-MS equipped with a collision cell to minimize mass interference. The analytical conditions were set as follows: a radio frequency power of 1500 W, radio frequency matching at 2 V, and a nebulizer gas (Ar) flow rate of 1.05 L/min. The limits of detection (LOD; mg/kg) for the UBM were as follows: As, 0.001; Ba, 0.001; Cd, 0.001; Cr, 0.001; Cu, 0.013; Ni, 0.001; Pb, 0.001; Sb, 0.003; Se, 0.005; Sn, 0.007; Sr, 0.0005; and Zn, 0.006. Those for the 0.07 M HCl single extraction method were As, 0.001; Ba, 0.0005; Cd, 0.001; Cr, 0.0003; Cu, 0.007; Ni, 0.001; Pb, 0.001; Sb, 0.002; Se, 0.003; Sn, 0.003; Sr, 0.0003; and Zn, 0.003. The matrix effect was corrected by subtracting the analysis signal of a blank sample (i.e., digestive fluids without CRM for the UBM and 0.07 M HCl solution without CRM for the 0.07 M HCl single extraction method) from the analysis signal of each sample.

Bioaccessibility was calculated as the percentage of the analysis result (CRM_C , mg/kg) obtained from the in vitro experiment with respect to the metal content in the CRMs used (certified value; CRM_T , mg/kg) (Equation (1)).

$$\text{Bioaccessibility (\%)} = \frac{CRM_C}{CRM_T} \times 100 \quad (1)$$

2.4. Statistical Analyses

The bioaccessible concentrations determined using each test method (i.e., the UBM and 0.07 M HCl single extraction) were expressed as the mean and standard deviation of triplicates. The average concentrations of all the metals ($n = 12$) leached from all the CRMs ($n = 13$) used in the experiments were compared for the two test methods.

Student's *t*-test was performed to examine the significant differences between the bioaccessibility results obtained using the UBM and 0.07 M HCl single extraction method; all significance levels were evaluated based on a two tailed *p*-value of 0.05. Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA) was used for the statistical analysis.

3. Results and Discussion

Figure 2 shows an excellent linear relationship of the all the metal/metalloid bioaccessibility results determined using the UBM and 0.07 M HCl single extraction ($R^2 = 0.82$) method. The slope of the linear regression was 1.38 (Figure 2), indicating that the 0.07 M HCl single extraction has potential for conservative assessment. The bioaccessible concentrations of all the metals/metalloids in all the CRMs determined using the UBM and the 0.07 M HCl single extraction method ranged from 0.002 to 17,449 mg/kg and from 0.003 to 20,283 mg/kg, respectively (Table 3). The bioaccessibility obtained with the UBM and the 0.07 M HCl single extraction method ranged from 0.00002 to 26.9% and from 0.00002 to 36.6%, respectively (Table 4). Overall, the bioaccessible metal/metalloid concentration and the bioaccessibility obtained using the 0.07 M HCl single extraction method were slightly higher than those obtained using the UBM. However, the results of Student's *t*-test using the two methods, for all the metals/metalloids, did not show a statistically significant difference ($p = 0.24$). Pelfrène et al. [17] confirmed that the 0.65% HCl extraction method could be used to effectively replace the UBM when testing 201 metal contaminated soils, and the R^2 values of the linear correlations for As, Cd, and Pb were 0.91, 0.97, and 0.72, respectively. Based on these results, the HCl single extraction method has been used to

determine metal bioaccessibility in soil [21,22]. Rodrigues et al. [19] also attempted to develop a single extraction method using 0.43 M HNO₃ as an alternative to the UBM and observed that the bioaccessibility values of Ba, Cd, Cu, Ni, Pb, and Zn in 204 soil samples assessed using the UBM were highly correlated with those obtained using the 0.43 M HNO₃ extraction method ($R^2 = 0.82\text{--}0.94$). Similarly, the relatively simple 0.07 M HCl single extraction method can be used as an in vitro test method to assess the oral bioaccessibility of metals/metalloids in various consumer products, replacing the UBM.

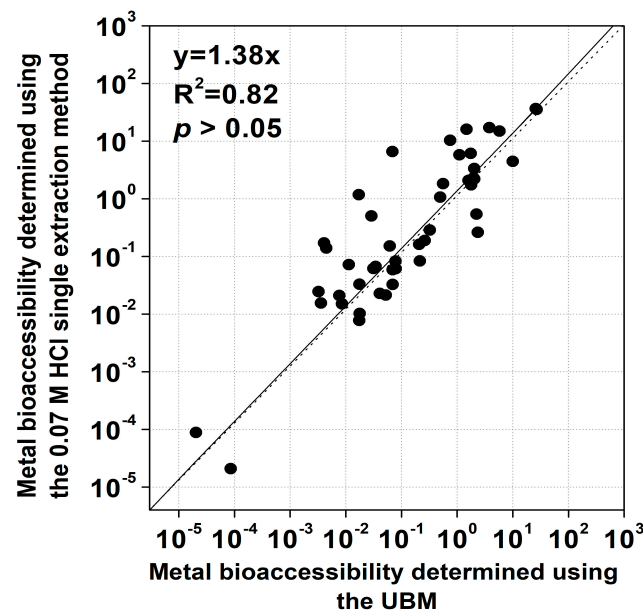


Figure 2. A linear relationship between the metal bioaccessibility results determined using the UBM and the 0.07 M HCl single extraction method. The dotted and solid lines indicate the 1:1 line and the linear regression, respectively.

To confirm the difference in the bioaccessibility of the metals/metalloids according to the material properties of the consumer products, the 13 CRMs were classified into 4 types: plastic, paint, metal, and glass. The results of the two bioaccessibility determination methods were compared for each of the classified CRM material properties (in the case of glass, the bioaccessibility evaluation result was only one; therefore, a linear relationship could not be confirmed) (Figure 3d–f). The linear regression slopes were 0.84 ($R^2 = 0.92$) for plastics (Figure 3d), 1.39 ($R^2 = 0.98$) for paints (Figure 3e), and 3.66 ($R^2 = 0.59$) for metals (Figure 3f), and no statistically significant differences were observed (all p -values > 0.05). The average bioaccessibility of the CRMs made of plastics, paints, and metals determined using the UBM was shown to be $0.69 \pm 2.00\%$, $12.1 \pm 13.2\%$, and $0.99 \pm 1.13\%$; however, when using the 0.07 M HCl single extraction method, bioaccessibility values of $0.39 \pm 0.87\%$, $17.98 \pm 17.5\%$, and $4.94 \pm 5.52\%$ were observed (Figure 3; Table 4). In particular, the metals and metalloids contained in paints are easily leached as compared to other materials. Further in-depth research on this topic is required. Additionally, the linear relationships between the analytical results obtained using the two the methods were compared for each element contained in the CRMs (Figure 3a–c). The linear regression slopes were 1.39 ($R^2 = 0.90$) for Cd (Figure 3a), 1.41 ($R^2 = 0.99$) for Cr ((Figure 3b), and 3.04 ($R^2 = 0.63$) for Pb (Figure 3c), and no statistically significant differences were observed (all p -values > 0.05). Similar trends were confirmed in the evaluation results for the other metals and metalloids (As, Ba, Cu, Ni, Sb, Sr, and Zn), except for Se and Sn (no individual figure is presented due to the lack of data ($n < 3$)).

Table 3. Bioaccessible metal concentrations in CRMs determined using the UBM and the 0.07 M HCl single extraction method.

CRM	Material	Method	Bioaccessible Concentration (mg/kg)											
			As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Se	Sn	Sr	Zn
JSM P701-1	PE	UBM	0.03 ± ^a 0.01	- ^b	0.04 ± 0.001	2.06 ± 0.43	-	-	0.07 ± 0.001	-	-	-	-	-
		0.07 M HCl	0.02 ± 0.002	-	0.08 ± 0.009	2.00 ± 1.16	-	-	0.17 ± 0.03	-	-	-	-	-
CRM 113-01-013	ABS	UBM	0.43 ± 0.04	-	0.01 ± 0.001	0.10 ± 0.01	-	0.07 ± 0.02	0.04 ± 0.004	<0.003	-	-	-	
		0.07 M HCl	0.38 ± 0.03	-	<0.001 ^c	0.08 ± 0.004	-	<0.001	0.08 ± 0.01	0.01 ± 0.004	-	-	-	
JSAC 0602-3	PET	UBM	-	-	0.01 ± 0.002	0.08 ± 0.01	-	-	0.02 ± 0.001	-	-	-	-	
		0.07 M HCl	-	-	0.04 ± 0.004	0.04 ± 0.03	-	-	0.04 ± 0.003	-	-	-	-	
ERM EC680m	PE	UBM	<0.001	-	0.002 ± 0.0003	0.03 ± 0.001	-	-	<0.001	0.01 ± 0.002	-	<0.007	-	4.59 ± 1.32
		0.07 M HCl	0.02 ± 0.001	-	0.003 ± 0.0004	0.02 ± 0.002	-	-	0.01 ± 0.001	0.01 ± 0.0004	-	0.07 ± 0.001	-	0.51 ± 0.03
ERM EC681m	PE	UBM	<0.001	-	0.01 ± 0.002	0.09 ± 0.05	-	-	<0.001	0.01 ± 0.0003	-	<0.007	-	<0.006
		0.07 M HCl	0.04 ± 0.003	-	0.02 ± 0.001	0.07 ± 0.002	-	-	0.05 ± 0.001	0.02 ± 0.0004	-	0.06 ± 0.004	-	0.53 ± 0.01
SRM 2861	PVC	UBM	0.01 ± 0.01	11.9 ± 1.01	0.05 ± 0.03	-	<0.013	-	0.04 ± 0.04	0.34 ± 0.05	0.52 ± 0.09	130 ± 7.06	-	-
		0.07 M HCl	0.06 ± 0.003	15.5 ± 0.44	0.04 ± 0.001	-	0.36 ± 0.04	-	0.02 ± 0.001	0.72 ± 0.08	0.20 ± 0.02	57.7 ± 3.13	-	-
BRM S004	Glass	UBM	-	-	-	0.08 ± 0.01	-	-	-	-	-	-	-	-
		0.07 M HCl	-	-	-	0.04 ± 0.01	-	-	-	-	-	-	-	-
110-05-paint-02	Paint	UBM	-	-	0.07 ± 0.01	-	-	-	19.2 ± 2.89	-	-	-	-	-
		0.07 M HCl	-	-	1.14 ± 0.30	-	-	-	20.9 ± 5.96	-	-	-	-	-
CRM 013-50G	Paint	UBM	-	-	10.2 ± 0.90	161 ± 6.31	-	-	37.1 ± 2.47	-	-	-	-	-
		0.07 M HCl	-	-	13.5 ± 2.09	226 ± 39.8	-	-	96.0 ± 19.8	-	-	-	-	-
SRM856a	Aluminum	UBM	-	-	-	<0.001	<0.013	0.15 ± 0.05	0.58 ± 0.06	-	-	<0.007	3.16 ± 0.92	6.60 ± 4.37
		0.07 M HCl	-	-	-	80.4 ± 1.54	48.9 ± 7.92	6.35 ± 0.25	0.24 ± 0.01	-	-	2.08 ± 0.34	11.0 ± 0.14	636 ± 23.2
SRM 872	Phosphor bronze	UBM	-	-	-	-	4920 ± 81	-	610 ± 33	-	-	7.14 ± 0.37	-	440 ± 11.4
		0.07 M HCl	-	-	-	-	16,036 ± 1748	-	6617 ± 80.7	-	-	491 ± 100	-	2313 ± 97.4
SRM 875	Cupronickel	UBM	-	-	0.84 ± 0.17	-	17,449 ± 220	2128 ± 23.5	0.69 ± 0.17	-	0.09 ± 0.01	<0.007	-	<0.006
		0.07 M HCl	-	-	3.76 ± 0.22	-	20,283 ± 1290	3484 ± 176	9.55 ± 0.37	-	0.02 ± 0.005	5.75 ± 1.50	-	79.8 ± 6.50
SRM 899	Nickel, Alloy	UBM	-	-	-	-	-	-	0.01 ± 0.002	-	0.08 ± 0.03	-	-	-
		0.07 M HCl	-	-	-	-	-	-	0.03 ± 0.003	-	0.02 ± 0.003	-	-	-

^a ±: standard deviations of triplicates. ^b -: elements not contained in the CRM. ^c <: limit of detection (LOD; mg/kg).

Table 4. Bioaccessibility of metals in CRMs determined using the UBM and the 0.07 M HCl single extraction method.

CRMs	Material	Methods	Bioaccessibility (%)											
			As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Se	Sn	Sr	Zn
JSM P701-1	PE	UBM	0.02	- ^a	0.03	1.80	-	-	0.06	-	-	-	-	-
		0.07 M HCl	0.01	-	0.07	1.74	-	-	0.15	-	-	-	-	-
CRM 113-01-013	ABS	UBM	0.32	-	0.10	0.07	-	0.05	0.03	<0.001	-	-	-	-
		0.07 M HCl	0.29	-	<0.004 ^b	0.06	-	<0.0003	0.06	0.005	-	-	-	-
JSAC 0602-3	PET	UBM	-	-	0.01	0.07	-	-	0.02	-	-	-	-	-
		0.07 M HCl	-	-	0.07	0.03	-	-	0.03	-	-	-	-	-
ERM EC680m	PE	UBM	<0.021	-	0.01	0.26	-	-	<0.01	0.08	-	<0.005	-	2.36
		0.07 M HCl	0.37	-	0.02	0.19	-	-	0.06	0.08	-	0.32	-	0.26
ERM EC681m	PE	UBM	<0.006	-	0.004	0.21	-	-	<0.001	0.01	-	<0.001	-	<0.0001
		0.07 M HCl	0.23	-	0.02	0.16	-	-	0.07	0.02	-	0.06	-	0.05
SRM 2861	PVC	UBM	0.003	1.61	0.08	-	<0.002	-	0.04	0.50	0.21	10.0	-	-
		0.07 M HCl	0.02	2.09	0.06	-	0.72	-	0.02	1.07	0.08	4.46	-	-
BRM S004	Glass	UBM	-	-	-	0.02	-	-	-	-	-	-	-	-
		0.07 M HCl	-	-	-	0.01	-	-	-	-	-	-	-	-
110-05-paint-02	Paint	UBM	-	-	0.03	-	-	-	2.03	-	-	-	-	-
		0.07 M HCl	-	-	0.50	-	-	-	2.22	-	-	-	-	-
CRM 013-50G	Paint	UBM	-	-	26.9	26.0	-	-	5.77	-	-	-	-	-
		0.07 M HCl	-	-	35.6	36.6	-	-	14.9	-	-	-	-	-
SRM 856a	Aluminum	UBM	-	-	-	<0.0002	<0.000003	0.004	0.05	-	-	<0.0001	1.76	0.07
		0.07 M HCl	-	-	-	13.4	0.14	0.17	0.02	-	-	0.21	6.11	6.62
SRM 872	Phosphor bronze	UBM	-	-	-	-	0.56	-	1.48	-	-	0.02	-	1.10
		0.07 M HCl	-	-	-	-	1.84	-	16.0	-	-	1.18	-	5.78
SRM 875	Cupronickel	UBM	-	-	3.81	-	1.99	2.04	0.75	-	2.23	<0.001	-	<0.0001
		0.07 M HCl	-	-	17.1	-	2.31	3.34	10.4	-	0.55	6.38	-	7.25
SRM 899	Nickel, Alloy	UBM	-	-	-	-	-	-	0.00002	-	0.0001	-	-	-
		0.07 M HCl	-	-	-	-	-	-	0.0001	-	0.00002	-	-	-

^a -: elements not contained in the CRM. ^b <: limit of detection (LOD; mg/kg).

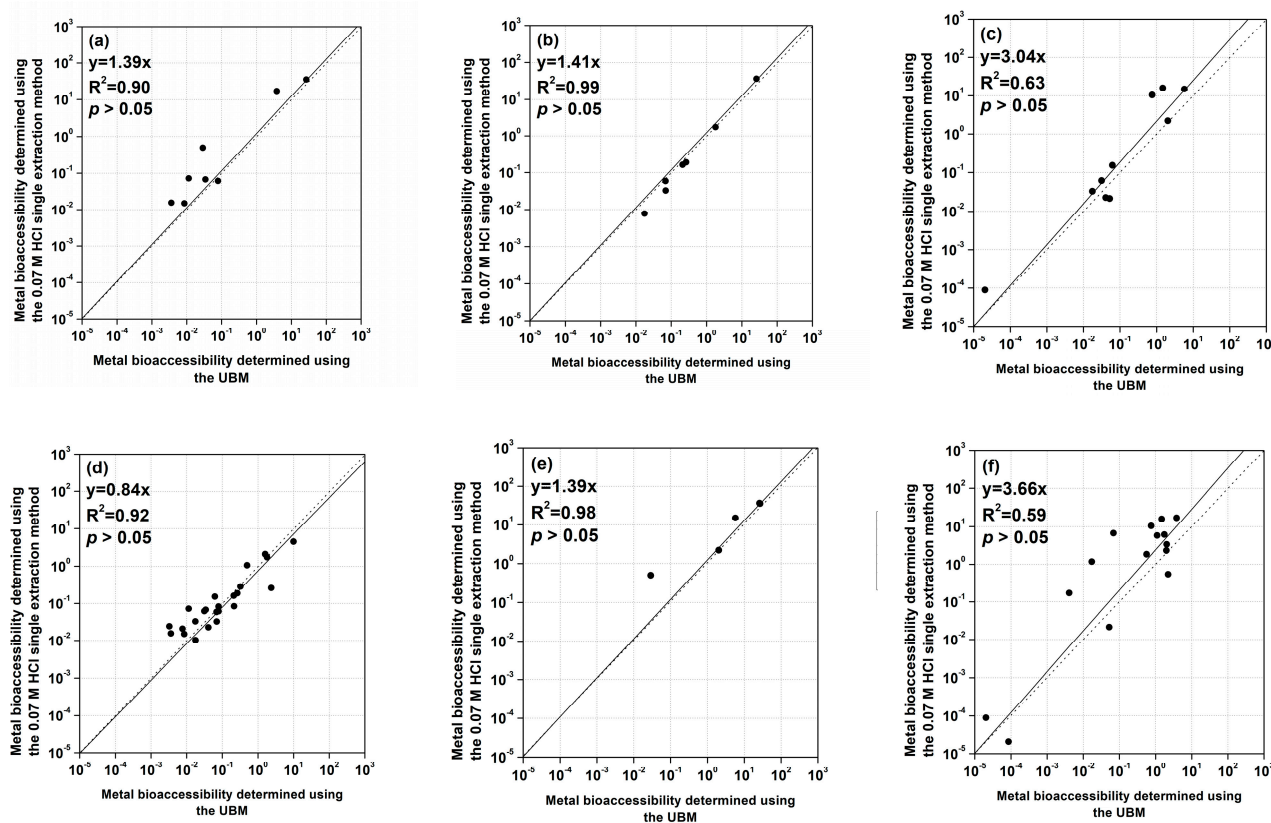


Figure 3. Linear relationships between the metal bioaccessibility determined using the UBM and the 0.07 M HCl single extraction method: (a) Cd, (b) Cr, (c) Pb, (d) plastics, (e) paints, and (f) metals. The dotted and solid lines indicate the 1:1 line and linear regression, respectively.

Meanwhile, artificial digestive fluid composition (ingredients) can affect the bioaccessibility of target metals and metalloids. The artificial digestive fluids of various components (pepsin, mucin, etc.) used for UBM testing contribute to the formation of metal complexes [21–25]. Metals extracted from low-pH gastric juice can interact (e.g., metal complex formation) with enzymatic components in the intestinal environment (i.e., neutral pH). These metal complexes may precipitate during centrifugation and be separated from the supernatant. They may also show a low ionization efficiency, even when introduced into analytical equipment (e.g., ICP-MS) [26]. Consistent with these observations, significant differences were observed in the bioaccessibility results obtained with the UBM and 0.07 M HCl single extraction method for Cr (UBM: <0.0002%, 0.07 M HCl: 13.4%) and Cu (UBM: <0.000003%, 0.07 M HCl: 0.14%) in SRM 856a and Zn in SRM 875 (UBM: <0.0001%, 0.07 M HCl: 7.25%). Conversely, Se in SRM 2861 (UBM: 0.21%, 0.07 M HCl: 0.08%) and Sn (UBM: 10.0%, 0.07 M HCl: 4.46%) and Zn (UBM: 2.36%, 0.07 M HCl: 0.26%) in ERM EC680m showed higher bioaccessibility using the UBM. Further research is needed to understand how the UBM facilitates metal extraction from certain products.

Although the specific interactions and mechanisms between the enzymatic components in artificial digestive fluids and target metals and metalloids are not well-understood, this study indicates that the bioaccessibility results determined using the two analytical methods are similar, regardless of these interactions ($p > 0.05$). In previous studies, the IVIVC values obtained from animal models and the UBM were higher than the validation criterion (i.e., $R^2 \geq 0.6$) while indicating the suitability of the UBM as an in vitro oral bioaccessibility test method for metal-contaminated soil [27,28]. Furthermore, Li et al. [29] confirmed that the UBM showed a higher IVIVC value than other in vitro bioaccessibility test methods (i.e., IVG [8], PBET [9], solubility bioaccessibility research consortium method (SBRC) [10], and DIN [12]) when assessing As-contaminated soils.

The UBM is an effective in vitro test method that can reasonably be used to assess the concentration of metal absorbed in the body (which can replace a bioassay), and its relative complexity can be overcome with a single extraction method, as described in this study (i.e., the 0.07 M HCl extraction method). It has the advantages of eliminating time-consuming and complicated procedures involved in the preparation of the UBM extractants and the dilution of eluates (i.e., to lower the high total dissolved solid content of the eluates) and can minimize the use of solvents in the pursuit of green analytical chemistry.

This study contributes to the establishment of a more reasonable human health risk assessment by presenting the alternative possibility of an in vitro bioaccessibility test method partially confirmed for metal-contaminated soil, introduced into the realm of consumer products for the first time. In particular, the 0.07 M HCl single extraction method can be used as a screening method prior to the application of the UBM. If a certain threshold is exceeded when applying 0.07 M HCl single extraction to consumer products, the use of the UBM to assess more realistically bioaccessibility can be considered.

4. Conclusions

In this study, the oral bioaccessibility of As, Ba, Cd, Cr, Cu, Ni, Pb, Sb, Se, Sn, Sr, and Zn in 13 CRMs was assessed using the UBM, as a precise means to mimic the human digestive processes, and the 0.07 M HCl single extraction method, as a simplified means. The bioaccessible metal concentrations evaluated using the UBM and the 0.07 M HCl single extraction method ranged from 0.002 to 17,449 mg/kg and from 0.003 to 20,283 mg/kg, respectively; their bioaccessibility ranged from 0.00002 to 26.9% and from 0.00002 to 36.6%, respectively. The 0.07 M HCl single extraction method showed relatively high concentrations, as the bioaccessible metal concentrations differed by 1.38 times (i.e., the slope of the linear regression), indicating its potential for conservative assessment. No statistically significant difference was observed between the results obtained using the two methods. This suggests that the 0.07 M HCl single extraction method can be used as an in vitro test method to assess the oral bioaccessibility of metals in a variety of consumer products, replacing the UBM and/or being used as a screening method prior to the application of the UBM, while eliminating the time-consuming procedures for preparing extractants and minimizing the use of solvents in order to adhere to green analytical chemistry practices.

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