UBM procedure for the measurement of inorganic contaminant bioaccessibility from solid matrices

1. INTRODUCTION

This document outlines the procedure to be followed for the measurement of inorganic contaminant bioaccessibility from soil according to the *in vivo* validated Unified BARGE Method. This method may be extended to sediments, vegetables, dusts, ash, or any other matrix studied in an exposure assessment approach, assuming the similar behavior of the test sample in the matrix to the soils used in the validation study.

2. REFERENCES

- Caboche, J. (2009). Validation d'un test de mesure de bioaccessibilité - Application à 4 éléments traces métalliques dans les sols : As, Cd, Pb et Sb. Laboratoire unité de recherche animale et fonctionnalités des produits animaux (URAFPA). Nancy, Institut national polytechnique de Lorraine.
- Denys, S., Tack, K., Caboche, J. & Delalain, P. (2008). Bioaccessibility, solid phase distribution, and speciation of Sb in soils and digestive fluids. *Chemosphere,* 74**,** 711-716.
- Denys, S., Caboche, J., Feidt, C., Hazebrouck, B., Dor, F., Dabin, C., Floch-Barneaud, A., & Tack, K. (2009). Biodisponibilité et bioaccessibilité des métaux et métalloides des sols pollués pour la voie orale chez l'homme. *Environnement, Risques et santé,* 8, (5), 433-438.
- Wragg, J, Cave, M, Taylor, H, Basta, N, Brandon, E, Casteel, S, Denys, S, Gron, C, Oomen, A, Reimer, K, Tack, K, and Van de Wiele, T. 2009. Interlaboratory Trial of a Unified Bioaccessibility Procedure. British Geological Survey, OR/07/027.
- Wragg, J. 2009. BGS Guidance Material 102, Ironstone Soil, Certificate of Analysis. British Geological Survey IR/09/006.

3. DEFINITIONS

3.1. TERMS

- Unified BARGE Method is an *in vitro* method for simulating the human digestive procedure using synthetic digestive fluids.
- Digestive fluids are synthetic fluids used in the UBM test to simulate the fluids present in human digestive system: saliva, gastric fluid, duodenal fluid and bile.
- Inorganic solutions containing inorganic salts (such as KCl, NaCl…) are used for the preparation of each digestive fluid.

- Organic solutions containing organic compounds (such as urea, glucose…) are used for the preparation of each digestive fluid.
- The gastric phase is a digestive extract collected after 1 hour agitation with saliva and gastric fluids.
- The gastro-intestinal phase is a digestive extract collected after 1 hour agitation with saliva and gastric fluid followed by 4 hours agitation with duodenal fluid and bile.

3.2. LIST OF ABBREVIATIONS

UBM	Unified BARGE method
BARGE	Bioaccessibility research group of Europe
S	Saliva
G	Gastric fluid
D	Duodenal fluid
B	Bile
I	Inorganic solution for the preparation of each digestive fluid
O	Organic solution for the preparation of each digestive fluid
Gc	Gastric phase of the UBM test
Ist	Gastro-intestinal phase of the UBM test
NaOH	Sodium hydroxide
HCl	Hydrochloric acid (37% Analytika Ltd.)
HNO_3	Nitric acid (67% Analytika Ltd.)

4. EXPERIMENTAL PROTOCOL

4.1. PRINCIPLE

The current procedure describes a method for simulating the human gastro-intestinal tract through 3 different compartments: mouth (5 minutes), stomach (1 hour) and small intestine (4 hours). Four digestive fluids are synthesized: saliva (S), Gastric fluid (G), duodenal fluid (D) and bile (B). Their composition is presented in section 7.3. All samples are mixed by end-over-end agitation at 37°C (human body temperature). The generalized UBM procedure is shown in Figure 1.

4.2. EQUIPMENTS

To carry out the UBM test, some specific equipment are required:

- polycarbonate tubes with caps
- oven/hot-plate at 37°C
- pH-meter
- "End-over-end" agitator/rotator at 37°C

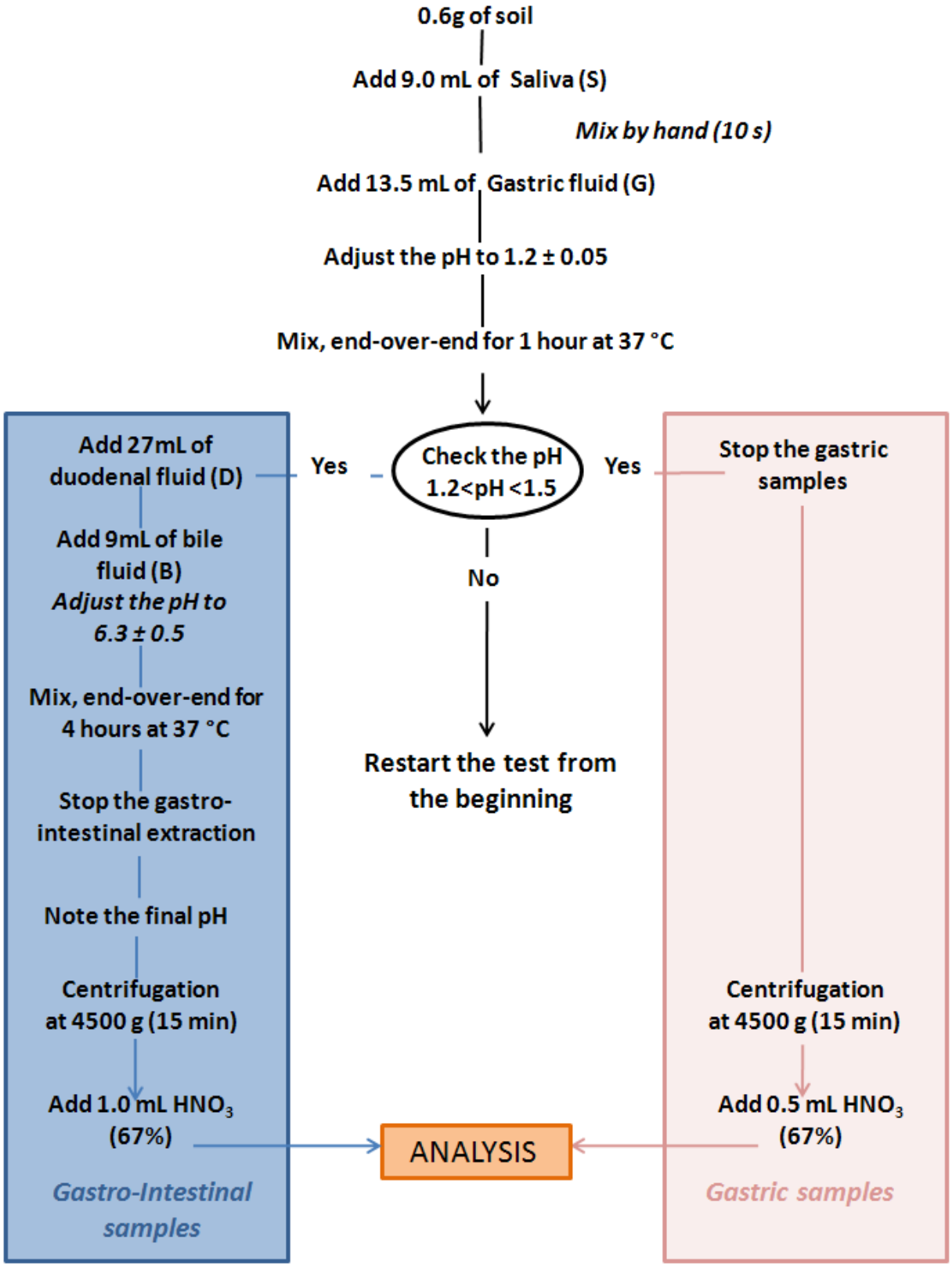

Figure 1 **Schematic diagram of the UBM methodology**

4.3. REAGENTS

Table 1 shows the various reagents used in the synthesis of the digestive fluids. All pH adjustments are performed with HCl 37% or NaOH 1-5 M purchased from Analytika Ltd or other suitable supplier.

Table 1 Reagents involved in the synthesis of the digestive fluids.

Reagents	Supplier	Product Code	CAS N°
NaH_2PO_4	Merck	1.06342.0250	13472-35-0
NaCl	Prolabo	27810.262	7647-14-5
KSCN	Sigma	P2713	333-20-0
Na_2SO_4	VWR	28114.230	7757-82-6
KCl	Merck	1.04936.1000	7447-40-7
$CaCl_2.2H_20$	VWR	1.02382.0250	10035-04-8
NH_4Cl	Prolabo	21236.291	12125-02-9
$NaHCO_3$	Prolabo	27778.293	144-55-8
KH_2PO4	Prolabo	26936.236	7778-77-0
$MgCl_2.6H_20$	Sigma	M8266	7786-30-3
NaOH	Prolabo	28244.295	1310-73-2
HCl	Analytika Ltd.		
Urea	Merck	108487	57-13-6
D + Glucose	VWR	101174Y	50-99-7
D – Glucuronic acid	Sigma	49335	6556-12-3
D-glucosaminehydrochloride	Sigma	G4875	66-84-2
Pepsine (porcine)	Merck	107185	9001-75-6
Bovine Serum Albumen	Merck	112018	90604-29-8
Mucin (porcine)	Sigma	M2378	84082-64-4
Uric Acid	Sigma	U2625	69-93-2
Pancreatin (porcine)	Merck	107130	8049-47-6
α-amylase (bacillus)	Sigma	A-6814	9000-90-2
Lipase (porcine)	Sigma	L-3126	9001-62-1
Bile (bovine)	Sigma	B-3883	8008-63-7

4.4. SAMPLE PREPARATION

The UBM test is carried out on 0.6 g of dried and sieved (<250 µm) samples. For each solid sample, two bioaccessible extracts are collected: one at the end of the gastric phase and another at the end of the gastro-intestinal phase. For each solid sample, the UBM test is performed in duplicate. Therefore for every solid sample, 4 liquid samples will be generated (2 replicates of the gastric phase and 2 replicates of the gastro-intestinal phase).

- Label the cleaned, dry polycarbonate tubes with
  - “Gq” for the gastric samples and “Ist” for the gastro-intestinal samples

- "1" or "2" referring to the replicate
- Name of the solid sample

Weigh 4 x 0.6 g replicates of each test soil into individual polycarbonate extraction tubes (2 replicates for the gastric phase and 2 replicates for the gastro-intestinal phase).

Two reference materials must be extracted within every batch of 10 unknown samples (corresponding to 20 samples in the gastric phase and 20 samples in the gastro-intestinal phase). Soil BGS-102 and NIST 2710a are useful and well referenced materials for the bioaccessibility of Arsenic, Lead and Cadmium.

4.5. pH-METER CALIBRATION

pH is an important parameter of the UBM test controlling the leaching of contaminants from the matrix. It must be fixed at 1.20 (± 0.05) during the gastric phase and 6.3 (±0.5) for the gastro-intestinal phase, thus the pH-meter must be precisely calibrated.

4.6. PREPARATION OF THE DIGESTIVE FLUIDS

Four digestive fluids are required for the UBM test: saliva (S), gastric (G), duodenal (D) and bile (B). All digestive fluids are prepared the day prior to their use in the UBM extraction procedure. The preparation of each fluid requires the preparation of two solutions (one inorganic (I) and one organic solution (O)). Each fluid (S, G, D or B) results from the combination of one inorganic solution, one organic solution and specific enzymes. Table 2 summarises the weight (mg) or volume (ml) of each reagent required for the preparation of 250 mL of each solution (I and O) for a final volume of each fluid about 500 mL (I+O). Once the I, O and enzymes are combined, place each fluid under magnetic agitation for at least 3 hours. Then, check the pH referring to pH values given in table 2 for each fluid. If necessary, adjust pH to the correct tolerance with NaOH 1M and/or HCl 37%. On the day of extraction, all fluids are placed at 37°C for at least one hour before the beginning of the UBM procedure.

Table 2 Masses and volumes of reagents for the preparation of 500 mL of each fluid (S, G, D, B) with 250 mL of inorganic solution (I) and 250 mL of organic solution (O)

	REAGENTS	Saliva (S)	Gastric (G)	Duodenal (D)	Bile (B)	Volume (mL)
Inorganic (I)	KCl	448	412	282	188	250
	NaH_2PO_4	444	133	-	-	
	KSCN	100	-	-	-	
	Na_2SO_4	285	-	-	-	
	NaCl	149	1376	3506	2630	
	$CaCl_2$	-	200	-	-	
	NH4Cl	-	153	-	-	
	$NaHCO_3$	-	-	2803.5	2893	
	KH_2PO_4	-	-	40	-	
	$MgCl_2$	-	-	25	-	
	NaOH (1M)	0.9 mL	-	-	-	
	HCl (37%)	-	4.15 mL	90 uL	90 uL	
Organic (O)	Urea	100 mg	42.5	50	125	250
	Glucose		325	-	-	
	Glucuronic acid		10	-	-	
	Glucosamine hydrochloride		165	-	-	
Enzymes	Alpha amylase	72.5 mg	-	-	-	250+250= 500
	Mucin	25 mg	1500	-	-	
	Uric acid	7.5 mg	-	-	-	
	Bovine Serum Albumin	-	500	500	900	
	Pepsin	-	500	-	-	
	$CaCl_2$	-	-	100	111	
	Pancreatin	-	-	1500	-	
	Lipase	-	-	250	-	
	Bile	-	-	-	3000	
pH	I+O	6,5 +/- 0,5	1,1 +/- 0,1	7,4 +/-0,2	8 +/- 0 .2	

4.7. GASTRO-INTESTINAL FLUID PH CONTROL

- Prepare an experimental gastric blank
  - Take one empty polycarbonate tube
  - Label the tube Gc_0
  - Add 9.0 mL of saliva (S)
  - Add 13.5 mL of gastric fluid (G)
  - Check the pH=1.20 ± 0.05
  - If necessary, adjust the pH in the gastric fluid (with NaOH 1M and/or HCl 37%) or in the saliva to obtain an experimental gastric blank at pH=1.20 ±0.05

- Prepare an experimental gastro-intestinal blank
    - Take one empty polycarbonate tube
    - Label the tube Ist_0
    - Add 9.0 mL of saliva (S)
    - Add 13.5 mL of gastric fluid (G)
    - Add 27.0 mL of duodenal fluid (D)
    - Add 9.0 mL of bile (B)
    - Check the pH=6.30 ±0.5
    - If necessary, adjust the pH in the duodenal fluid or in the bile (with NaOH 1M and/or HCl 37%) to obtain an experimental gastro-intestinal blank at pH=6.30 ±0.5

5. UBM TEST

For each polycarbonate tube:

1) Add 9.0 mL of saliva (S) by pipette. Quickly shake by hand (10 seconds).
2) Add 13.5 mL of gastric fluid (G) by pipette.
3) Check pH=1.20 ±0.05. Adjust with NaOH 1M and/or HCl 37% if necessary.
4) Quickly shake by hand (10 seconds).
5) Check pH=1.20 ±0.05. Adjust with NaOH 1M and/or HCl 37% if necessary.
6) Repeat this operation until the pH stay stable at 1.20 ±0.05
7) Place the tubes in the "end-over-end" rotator.
8) Place the agitator in a stove/heated water bath at 37°C for 1 hour
9) Remove the tubes from the stove/heated water bath.
10) Check the pH<1.50 (If not, the procedure should be restarted from the beginning with special insistence on the pH stability at 1.20 ±0.05.
11) Separate the gastric samples (Gc) from the gastro-intestinal samples (Ist)
    - Centrifuge the samples for 15 minutes at 4500 g.
    - Collect the supernatant by careful pipetting
    - Acidify the extract with 500 µL HNO_3
    - Analyse the gastric samples by ICP MS/AES
12) The following steps are applied to the gastro-intestinal samples only
13) Add 27 mL of duodenal fluid in the gastro-intestinal tubes (Ist)
14) Add 9.0 mL of bile fluid by pipette
15) Check pH=6.30 ±0.5. Adjust with NaOH 1M and/or HCl 37% if necessary.
16) Place the tubes in the "end-over-end" rotator.
17) Place the agitator in a stove/heated water bath at 37°C for 4 hours.
18) Remove the tubes from the stove/heated water bath.
19) Note the final pH of the extracts.
20) Centrifuge the samples for 15 minutes at 4500 g.
    - Collect the supernatant by careful pipetting
    - Acidify the extract with 1.0 mL HNO_3

- Analyse the gastro-intestinal samples by ICP MS/AES

6. RESULTS

The results are expressed in mg of bioaccessible contaminant per kg of solid matrix. They are also expressed as a percentage of the bioaccessible contaminant concentration, calculated as follows:

$$\%\ bioaccessible = \frac{Concentration\ of\ bioaccessible\ metal\ (mg.kg^{-1})}{Concentration\ of\ total\ metal\ in\ sample\ (mg.kg^{-1})} \times 100$$

The concentration of the total contaminant in the sample is determined after digestion of the solid sample with aqua regia or other suitable mixed acid.

7. CLEANING

At the end of the test, the polycarbonate tubes are rinsed 3 times with de-ionized water and then filled with 2% HNO_3 until the next use.

Table 3 Quantification and detection limits in the UBM gastro-intestinal media determined with ICP-MS (Agilent 7500 CX). Data collected according to the analytical/instrumental conditions applied at INERIS and supplied for information only.

Element	Isotope	Isotopic abundance	Analysis mode	Units	UBM Blank	Minimal dilution	LQ	LD	Units	LQ	LD
Mg	24	79%	Without gas	$mg.L^{-1}$	5.701±0.225	100	0.500	0.167	$mg.kg^{-1}$	50	17
	26	11%		$mg.L^{-1}$	5.997±0.352	100	0.500	0.167	$mg.kg^{-1}$	50	17
Al	27	100		$\mu g.L^{-1}$	12.890±0.523	100	100	33	$mg.kg^{-1}$	50	17
Ti	46	8%	Helium	$\mu g.L^{-1}$	15.789±1.112	2	2	0.7	$mg.kg^{-1}$	0.2	0.1
	48	74%		$\mu g.L^{-1}$	111.825±1.272	2	5	1.7	$mg.kg^{-1}$	0.5	0.2
V	51	100%		$\mu g.L^{-1}$	1.144±0.018	2	1	0.3	$\mu g.kg^{-1}$	100	33
Cr	52	84%		$ng.L^{-1}$	929±18	2	500	167	$\mu g.kg^{-1}$	50	17
	53	10%		$\mu g.L^{-1}$	1.560±0.074	2	0.5	0.17	$\mu g.kg^{-1}$	50	17
Mn	55	100		$\mu g.L^{-1}$	10.440±0.128	2	10	3	$mg.kg^{-1}$	1	0.3
Fe	56	92%		$\mu g.L^{-1}$	155.203±3.647	100	100	33	$mg.kg^{-1}$	50	17
	57	2%		$\mu g.L^{-1}$	178.923±2.952	100	100	33	$mg.kg^{-1}$	50	17
Ni	58	68%		$\mu g.L^{-1}$	1.643±0.035	2	1	0.33	$\mu g.kg^{-1}$	100	33
	60	26%		$ng.L^{-1}$	945±20	2	200	67	$\mu g.kg^{-1}$	20	7
Co	59	100%		$ng.L^{-1}$	111±4	2	100	33	$\mu g.kg^{-1}$	10	3
Cu	63	69%		$\mu g.L^{-1}$	9.717±0.084	2	10	3	$mg.kg^{-1}$	1	0
	65	31%		$\mu g.L^{-1}$	9.583±0.119	2	10	3	$mg.kg^{-1}$	1	0
Zn	64	49%		$\mu g.L^{-1}$	140.306±1.169	100	25	8	$mg.kg^{-1}$	50	17
	66	28%		$\mu g.L^{-1}$	148.177±0.917	100	25	8	$mg.kg^{-1}$	50	17
	68	19%		$\mu g.L^{-1}$	131.791±1.614	100	25	8	$mg.kg^{-1}$	50	17
As	75	100%		$ng.L^{-1}$	184±7	2	200	67	$\mu g.kg^{-1}$	20	7
Se	80	50%	Hydrogen	$\mu g.L^{-1}$	11.146±0.129	2	2	0.7	$mg.kg^{-1}$	0.2	0.1
	82	9%		$\mu g.L^{-1}$	53.968±0.748	2	2	0.7	$mg.kg^{-1}$	0.2	0.1

Table 4 Quantification and detection limits in the UBM gastro-intestinal media determined with ICP-MS (Agilent 7500 CX). Data collected according to the analytical/instrumental conditions applied at INERIS and supplied for information only.

Element	Isotope	Isotopic abundance	Analysis mode	Units	UBM Blank	Minimal dilution	LQ	LD	Units	LQ	LD
Mo	92	15%	Without gas	$ng.L^{-1}$	991±31	2	1000	333	$mg.kg^{-1}$	0.1	0.03
	98	24%		$ng.L^{-1}$	274±7	2	500	167	$\mu g.kg^{-1}$	50	17
Ag	107	52%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
	109	48%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
Cd	111	13%		$ng.L^{-1}$	77±3	2	100	33	$\mu g.kg^{-1}$	10	3
	113	12%		$ng.L^{-1}$	0	2	1000	333	$mg.kg^{-1}$	0.1	0.03
	114	29%		$ng.L^{-1}$	0	2	100	33	$\mu g.kg^{-1}$	10	3
Sn	116	15%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
	120	33%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
Sb	121	57%		$ng.L^{-1}$	175±11	2	100	33	$\mu g.kg^{-1}$	10	3
	123	43%		$ng.L^{-1}$	197±16	2	100	33	$\mu g.kg^{-1}$	10	3
Te	126	19%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
	128	32%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
	130	34%		$ng.L^{-1}$	0	2	10000	3333	$mg.kg^{-1}$	1	0.3
Tl	203	30%		$ng.L^{-1}$	0	2	100	33	$\mu g.kg^{-1}$	10	3
	205	70%		$ng.L^{-1}$	0	2	200	67	$\mu g.kg^{-1}$	20	7
Pb	206	24%		$ng.L^{-1}$	334±4	2	100	33	$\mu g.kg^{-1}$	10	3
	207	22%		$ng.L^{-1}$	366±27	2	200	67	$\mu g.kg^{-1}$	20	7
	208	52%		$ng.L^{-1}$	306±8	2	100	33	$\mu g.kg^{-1}$	10	3