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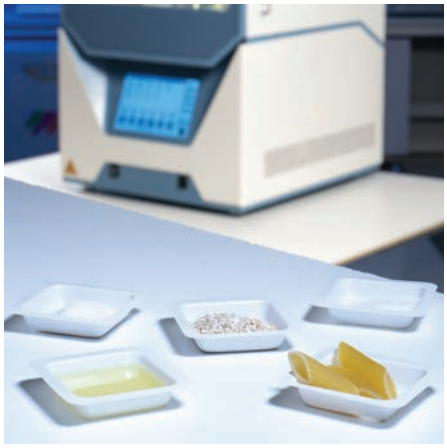

BEST PRACTICES IN SAMPLE PREPARATION **OF BABY FOOD FOR TRACE METAL DETERMINATION**

MILESTONE

HELPING CHEMISTS

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“This planet is all we have. If we spoil it, our children will have to pay to clean up the mess we make. Our own body is all we have. If we abuse it, or in our ignorance feed it badly, then one day there will be a price to pay. It is no longer good enough to say we can do nothing, that we must leave the wellbeing of our planet to those who claim to be its guardians. Similarly, when it comes to knowing about the minerals that our body needs, we cannot simply leave it to doctors and dieticians to correct things when they go wrong, because our eating habits are faulty.”

John Emsley, Nature’s Building Blocks.
An A-Z Guide to the Elements, Oxford University Press, Oxford, 2001

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Best Practices in Sample Preparation of Baby Food for Trace Metal Determination

1.

Baby food safety: the health impact of heavy metals and the importance of their trace determination

Food is the fuel for life and our health depends on our choices considering which foods we eat and also which foods we do not eat. To keep our body in balance requires a careful consideration of the quality and the quantity of our food choices. Of course, we are responsible for our choices and we can make better ones by having chemical and nutritional information. Food choices are dependent on availability, costs, cultural habits, etc., but it is also important to have information about proteins, fats, carbohydrates, and minerals content. There are several chemical elements that we need in high amounts, such as calcium, phosphorus, potassium, sulphur, sodium, chlorine, and magnesium. There are others that, despite being essential, we need in comparatively minor amounts, such as iron, zinc, copper, manganese, iodine, and selenium. Also, there are some elements that we must not ingest, such as arsenic, cadmium, lead, and mercury. Eliminating these latter elements as much as possible is critical for health of adults, and even more critical for the health of infants.

33
As

Arsenic is notorious as an element related to deadly poison [1]. Arsenic acts by blocking the action of some enzymes and symptoms of arsenic poisoning are vomiting, colic, diarrhea, and dehydration. High doses may cause heart failure and death.

48
Cd

Cadmium also interacts with enzymes, e.g., metallothionein, which contains sulphur and may accumulate in kidneys. If the levels become too high, the body's filtering system is damaged and kidney failure may occur. Continuous exposition to cadmium also weakens the bones and joints.

82
Pb

Toxic effects caused by lead are well-known and there is a long history of contamination caused by this element. For instance, there are historical descriptions of lead contamination in the ancient Greeks and Romans. Atkins presented an interesting discussion about lead in the food supply and she pointed out that Romans used lead in applications ranging from water pipes to tableware and cosmetics [2]. According to Whitney and Rolfes, "Like other minerals, lead is indestructible… Chemically similar to nutrient minerals like iron, calcium, and zinc, lead displaces them from some slots they normally occupy, but is then unable to perform their roles…
Lead damages many body systems, particularly the vulnerable nervous systems, kidneys, and bone marrow… The greater the exposure, the more damaging the effects." [3].

80
Hg

Finally, mercury is another ubiquitous toxic element with critical effects on several organs including the central nervous system. Mercury poisoning causes several physical symptoms including headache, nausea, vomiting, stomach pains, diarrhea, and a metallic taste in the mouth. According to Emsley, poisoning by smaller amounts over longer periods of time, i.e., chronic mercury exposure, causes fatigue, weakness, loss of memory and insomnia [4].

Toxicities of these elements are dependent on their chemical forms. For instance, it is well-known that inorganic forms of arsenic, e.g., As(III) and As(V), are more toxic than organoarsenic compounds, such as arsenobetaine and arseno-sugars. For mercury, all forms are toxic, but the most toxic forms are organomercury compounds, which caused the critical Minamata Bay disaster in Japan in the 1950s. How are we exposed to these critical elements? Based on lead contamination in earlier ages, this is not a new problem, but of course nowadays we have better education and a huge flow of information to help us to measure the extension of the problem and to tackle it.
It is not an exaggeration to say that these elements are everywhere and that they have had unexpected applications and technological uses. Arsenic uses through history range from medicines (Dr. Fowler's solution) to green wallpaper (Scheele's green – copper arsenite).
This latter compound was even used to colour sweets and make them more attractive [1].
Arsenic has also been widely used in agriculture. Cadmium is used in batteries and it is also a contaminant of some phosphate rocks used as fertilizers. Emsley mentioned that phosphate rock from Morocco contains over 50 g Cd per tonne [4]. Lead and arsenic are also found in many fertilizers. Unfortunately, lead and mercury are essentially everywhere. Lead is present in and can leach from old water pipes. You have certainly heard about

the lead crisis in the Flint water supply in 2014 in the United States [2]. Whitney and Rolfes affirmed hat "all foods contain some lead" and "lead poisoning in infants most often comes from infant formula made with contaminated water" [3]. They recommended that "The first water drawn from the tap each day is highest in lead – therefore, a person living in a house with old, lead-soldered plumbing should let the water run a few minutes before drinking or using it to prepare formula of food." [3]. It is also important to remember the long decades of use of tetraethyl lead in automotive gasoline and the use of lead carbonate as the base pigment for paints, coatings, and even cosmetics *(Figure 1)*.
Mercury is also broadly used and nowadays it is still used in the mining of gold. In summary, when we consider natural occurrence in the earth's crust, industrial uses, technological uses, and so on, it is not surprising to find these elements in our food chain. However, despite not being surprising, it is certainly worrisome. If we are used to reading about food safety, we know that rice may contain arsenic, cadmium may occur in cereals, and mercury compounds are common in fishes and seafoods. All these elements are critical if present in foods, and even worse if these foods are used to feed babies and infants. ***"The Baby Food Safety Act of 2021"***, a new bill of the United

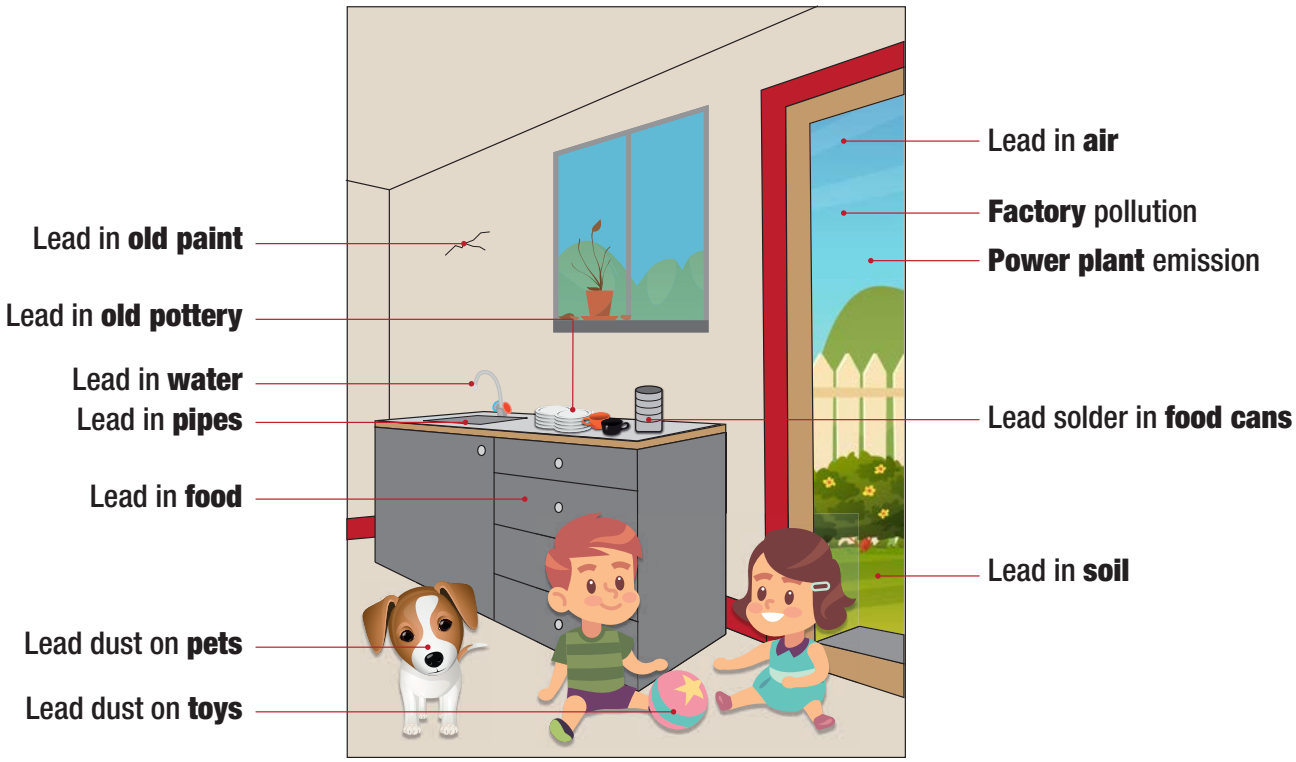

Figure 1. Potential sources of lead exposure

Modified version of the figure reported in reference [3]

States Congress, defines “infant and toddler food” as food intended for sale to children up to 36 months of age, including infant formula.”
It is stated that: “Inorganic arsenic, lead, cadmium, and mercury are toxic heavy metals. The Food and Drug Administration (FDA) and the World Health Organization have declared them dangerous to human health, particularly to babies and children, who are most vulnerable to their neurotoxic effects. Even low levels of exposure can cause permanent decreases in IQ, diminished future economic productivity, and increased risk of future criminal and antisocial behavior in children.”
According to an FDA scientist, since children approaching the age of 36 months are normally eating the same foods as adults, this Safety Act could apply to almost any kind of food.
Based on this background, it is established ***”The Baby Food Safety Act of 2021”*** would require manufacturers and the FDA to take long overdue action by:

- Setting maximum levels of inorganic arsenic (10 ppb, 15 ppb for cereal), lead (5 ppb, 10 ppb for cereal), cadmium (5 ppb, 10 ppb for cereal), and mercury (2 ppb) allowed in baby food that manufacturers would have to meet within one year.
- Requiring those levels to be lowered further within two years through FDA guidance, and again after three years through regulation.
- Requiring manufacturers to test their final products – not just ingredients – for toxic heavy metals (ingredient testing significantly underestimates toxic heavy metal levels).
- Requiring manufacturers to post the results of their product testing online twice per year.
- Establishing a public awareness campaign through the CDC to highlight the risks posed by toxic heavy metals in baby food.
- Authorizing $50 million for research on agricultural methods of reducing toxic heavy metals in crops.

At this stage, it is clear that we have two important allies for improving food safety: education and legislation. The next steps to act would logically include increasing information about food composition and, of course, determining the concentrations of these elemental contaminants. As established by the new legislation: “Require manufacturers to conduct representative testing of final products for toxic heavy metals as part of their hazard preventive control efforts” and to disseminate this information; “Require manufacturers to make publicly available online, twice per year, reports summarizing the results of their product testing and their efforts to monitor and verify the effectiveness of hazard preventive controls.” A classical quote citing Lord Kelvin says: “When you can measure what you are speaking about, and express it in numbers, you know something about it...” To measure is to know. According to the new legislation we must be able to measure concentrations of As, Cd, Hg, and Pb in levels from 2 to 15 µg/kg. It is also stated that these levels should go down: “Requiring those levels to be lowered further within two years through FDA guidance, and again after three years through regulation.” The Food and Drug Administration is working on a “closer to zero plan”. But, how “close to zero” can we realistically measure? How can we trust “close to zero” data? Nowadays, we may go forward building on Lord Kelvin’s aphorism: to measure accurately is to become able to act and to solve critical issues in all areas. When talking about the safety of baby foods, we are coping with an absolutely critical public health issue with clear implications in our future as a healthy society. Just as a reminder about how far science is moving us, in a note published in 1994 it was cited that the lead specification set by FDA in 1958 was 10 mg/kg (!!) and the new lead limits would be 0.5 mg/kg for food ingredients consumed in moderate amounts and 0.1 mg/kg for ingredients consumed in large amounts [5]. Analytical chemists have developed powerful strategies for determining trace elements. Of course, modern instrumental methods, e.g., inductively coupled plasma mass spectrometry, are important for meeting these analytical demands and their use is increasing considering, for instance, recent legislation related to contaminants in medicines [6]. However, despite having proper instrumentation available, we need to strengthen the analyst’s culture about working with trace concentrations and to educate the community about contamination sources in typical analytical procedures. As we have disseminated before, and we will stress here: “Think Blank” and keep careful control of all analytical steps [7].

Consequently, once again sample preparation will be shown as a fundamental step for obtaining accurate and precise results in trace analysis. Some items to which we must pay close attention include:

- Purity of reagents, as well as how they can be easily purified.
- Contamination and cleaning of laboratory materials.
- Sample preparation procedures that involve digesting high amounts of samples using low volumes of purified nitric acid.
- Analytical procedures with lower numbers of successive steps.

All these points will be highlighted in the following sections and we will demonstrate how optimized procedures can be successfully developed and applied to meeting the demands of a “closer to zero plan.”

2.
Sample preparation Part I: Clean Strategies

Attaining success in trace elemental analysis is closely related to the application of suitable sample preparation procedures performed with a thorough control of the analytical blank. Nowadays, best conditions for sample preparation for trace element analysis are established using microwave-assisted acid digestion performed in closed vessels or reaction chambers. Of course, we must be aware of all aspects involved in the analytical procedure, e.g., vessels for performing digestions should be built with suitable materials and they should be properly cleaned, pure reagents must be used, and contamination sources should be controlled. Tailored procedures should involve few analytical steps and ideally transference of solutions among vessels should be avoided, reduced, or simplified as much as possible. Furthermore, for determining elements present in increasingly low concentrations, an ideal analytical procedure should be capable of digesting high masses of samples using minimum volumes of nitric acid. Fortunately, several developments proposed in the last several years allow us to meet these demands and some critical aspects are discussed below. Highly purified concentrated acids are expensive, but we also know that these reagents can be easily purified in-lab using sub-boiling distillation [8]. This is a simple and effective procedure for producing high-purity reagents without requiring extensive involvement of specialized personnel *(Figure 2)*. As previously discussed [9], sub-boiling distillation uses contact-less infrared heating to vaporize the surface liquid at a temperature typically 20 °C below the boiling point of the reagent. Consequently, a gentle surface evaporation during sub-boiling distillation prevents the formation of spray or droplets from traditional boiling that could contain contaminants *(Figure 3)* and thus yields high-purity distillates *(Table 1)*. This procedure is easily applied to production of commonly used high-purity reagents, such as nitric and hydrochloric acids. Milestone has developed a convenient apparatus, named duoPUR, that is capable of taking reagent-grade HNO_3 and HCl and easily purifying them in-lab and on demand (a dedicated version for HF is also available, named subCLEAN).

Figure 2. Milestone duoPUR, sub-boiling distillation system

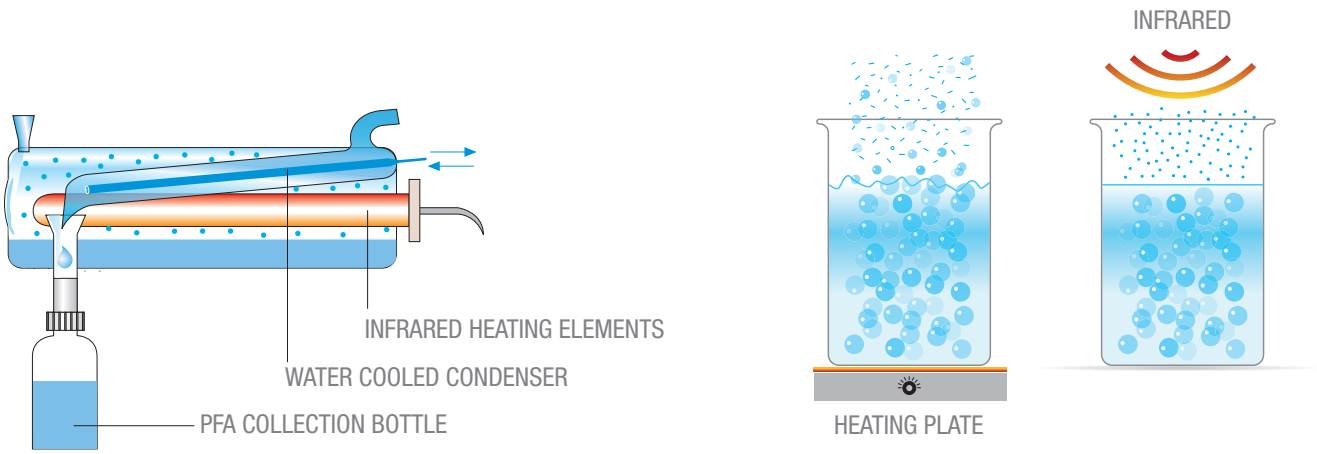

Figure 3. Boiling versus sub-boiling vaporization processes

Table 1. Trace metal contamination (ng/L) in nitric acid produced by sub-boiling distillation in a quartz still.

	Nitric Acid - ACS GRADE specification (ng/L)	*Nitric acid - Sub-boiling distillation (ng/L)*
As	< 10000	< 100
Ba	< 10000	< 50
Cd	< 10000	< 5
Cr	< 20000	< 20
Co	< 10000	< 5
Cu	< 10000	< 1000
Hg	< 10000	<10
Mn	< 10000	< 100
Mo	< 20000	< 10
Ni	< 20000	< 500
Pb	< 10000	< 20
Sb	< 10000	< 10
Se	< 10000	< 500
Sr	< 10000	< 500
Te	< 10000	< 50
V	< 10000	< 10
W	< 10000	< 10

duoPUR, depending on the operational conditions employed, produces 30 to 140 mL per hour of HNO_3 and 20 to 130 mL per hour of HCl, as shown in *Table 2*. We consider this equipment as essential for busy trace elemental analysis laboratories that require a steady supply of freshly purified acids. In addition to its simplicity, it is also worth pointing out that there are significant cost savings when using duoPUR when compared to purchasing commercially produced high-purity reagents [10]. This is demonstrated in the following example considering a laboratory applying the US

FDA EAM 4.7 method and taking into account the additional acids used for calibration standards, QC samples, sample dilution, and rinsing between samples (all costs are provided as estimates for example purposes only): If a laboratory processes 200 samples per month using an average of 18 mL of Ultrapur nitric acid (Merck ≥60%, €1520 per liter) per sample analyzed, it will use 3.6 L of ultra-pure acid at a cost of approximately €5,472 per month. Instead, reagent grade acid (Merck, reag. ISO, Ph. Eur) would only cost approximately €144 per month (€40 per liter) By distilling this lower grade acid, €5,328 would be saved every month.

Future perspective *- Recently, the possibility of repurifying contaminated acids was demonstrated by Mello et al. [11]. These authors have demonstrated how acid digests can be recycled and reused for further digestions without compromising accuracy of analyses. It was concluded that sub-boiling distillation minimizes both the use of acids and the generation of waste and it is fully compatible with green chemistry principles by reducing-recycling-reusing acids without using high amounts of either energy or manpower.*

The availability of high-purity acids being assured, the next steps are to select suitable digestion vessels that are compatible with the trace elemental analysis requirements, and to define the strategies to properly clean the digestion vessels in between digestion runs. Simple disposable vial-type vessels can be used for many routine analyses that do not have stringent requirements for determining trace elements. However, when dealing with trace analysis at the lowest concentration levels, the best results are attained using vessels constructed from quartz or TFM-PTFE. As a general principle in trace analysis, we can assume that all receptacles and ancillary pieces that come in contact with samples should be cleaned before use, but it is still important to perform blank measurements in all procedures to check for any contamination. Analyses of multiple blanks are as important as sample analysis when working with trace elemental analysis. Traditionally, digestion vessels are cleaned by immersion for about 12 h in an acid bath. However, this approach is often not sufficient when determining analytes at lower trace concentrations, such as the expected concentrations of As, Cd, Pb, and Hg contaminants in baby foods. The most effective cleaning of digestion vessels is accomplished by using acid vapor for leaching metal contaminants from the vessel walls. Milestone has developed practical equipment for performing this procedure

Table 2. duoPUR typical distillation rates for HNO_3 and HCl in mL/hour based on varying infrared power settings (expressed in percentage, double-boiler mode).

Sub-boiling distillation of 500 mL HNO_3					
Power	***20%***	***50%***	***55%***	***60%***	***65%***
Sub-boiling HNO_3 distillation rate	30 mL/h	100 mL/h	110 mL/h	130 mL/h	140 mL/h

Sub-boiling distillation of 500 mL HCl						
Power	***15%***	***30%***	***35%***	***45%***	***50%***	***60%***
Sub-boiling HCl distillation rate	20 mL/h	60 mL/h	64 mL/h	90 mL/h	110 mL/h	130 mL/h

in an automated manner called traceCLEAN *(Figure 4)*. Once the vessels are loaded into the system, the time and temperature required for the cleaning are simply entered on the traceCLEAN controller and freshly distilled acid vapor is then continuously refluxed within the sealed unit to clean the vessels. The use of traceCLEAN also results in a significant reduction of the acid consumption typically used for cleaning procedures. The system typically consumes approximately 500 mL of HNO_3 (technical grade) for 20 cleaning cycles, which collectively can clean over a thousand vials (calculation based on 15-mL ultraWAVE 3 vials).

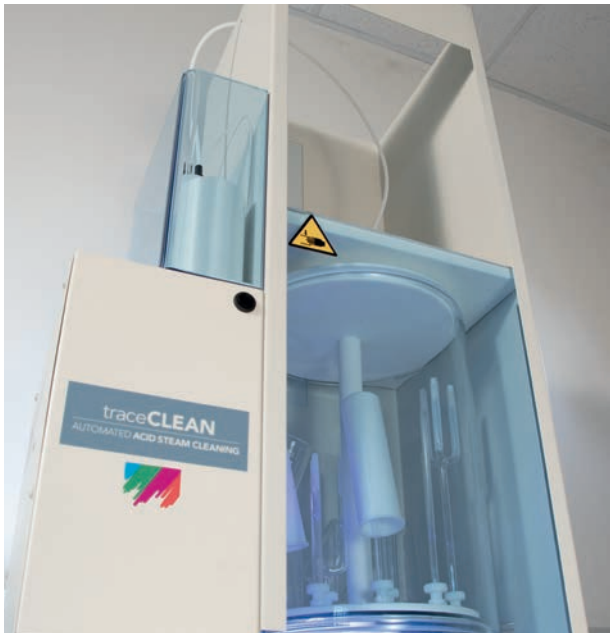

Figure 4. Milestone traceCLEAN - Acid steam cleaning

A practical laboratory routine was simulated to demonstrate the application of traceCLEAN for cleaning digestion vials and vessels in between sample batches. In this trial, milk powder (0.5 g), spiked at a high concentration level (1 mg/kg) with As, Cd, Pb, Hg, was digested in quartz and PTFE vials and vessels suitable for ultraWAVE 3 and ETHOS UP, respectively. After every digestion, the quartz and PTFE vials and vessels were cleaned using traceCLEAN. Blank measurements were then performed on the vials and vessels before they were used for further sample digestions. For the blank tests, 2.5 mL of HNO_3, 0.5 mL of HCl, and 1 mL of DI H_2O were added to the vials and vessels and then typical digestion heating cycles were performed in the microwave systems. Afterwards, once cooled, the solutions were brought to a final volume of 50 mL and analyzed using triple-quadrupole ICP-MS (*Table 3* and see *Appendix* for method details).

Another important aspect for optimizing trace analysis procedures is minimizing the analytical steps and limiting, as much as possible, of the risk of human error. For instance, simply reducing filtering, transferring, or more in general the number of handling steps, at minimum is beneficial for the proper control of the analytical blank.

The complete analytical preparation procedure should be evaluated, and any unneeded step should be avoided or simplified if possible. To help chemists optimize their procedures, Milestone developed the easyFILL system (*Figure 5*) for the automatic addition of acids during the preparation of digestion mixtures. easyFILL is capable of automatically adding any type of acid into the digestion vessels and vials, limiting as much as possible the manipulation of the digestion mixture and reducing the risk of possible contamination.

Figure 5. Milestone easyFILL - Automated Dosing Station

Table 3. Blank measurement using triple-quadrupole ICP-MS after traceCLEAN treatment (blank test was performed at 250 °C for ultraWAVE 3 and at 210 °C for ETHOS UP). Data reported in this table are average results of 5 repetitions for each test.

	As (µg/L)	***Cd (µg/L)***	***Pb (µg/L)***	***Hg (µg/L)***
ultraWAVE 3 - Quartz vial	<0.01	<0.01	<0.01	<0.005
ultraWAVE 3 - PTFE vial	<0.01	<0.01	<0.01	<0.005
ETHOS UP – MAXI 24 HP PTFE vessels	<0.01	<0.01	<0.01	<0.005

3.

Sample preparation Part II: Microwave-assisted sample preparation tailored for a “closer to zero plan”

The evolution of microwave-assisted sample preparation has brought significant advances in microwave instruments with higher applied power, full control of digestion conditions, and enhanced safety for analysts and the laboratory environment. These goals were attained by proper development of ovens and closed reaction vessels with temperature and pressure sensors that allow real-time monitoring of digestion conditions. Closed reaction vessels should have the following characteristics:

- Materials used should avoid sample contamination and memory effects.
- Materials should support high temperatures (around 260 °C) and high pressures (around 80-100 bar) without mechanical deformation and without affecting their microstructure stability (i.e., container walls should not become porous even after several heating-cooling cycles).
- Materials should be highly chemically resistant to concentrated hot acid mixtures.
- Design of the reaction vessels must guarantee full recovery of analytes and safe operation.
- Materials should be easily cleaned.

These requirements have been reached with modern closed vessels, the development of which satisfies a critical requirement for dealing with trace elemental analysis. We may consider a closed vessel as a micro-laboratory system isolated from the environment and which allows feasible conditions for trace analysis without compromising analytical blanks.
US FDA EAM (Elemental Analysis Manual) 4.7 describes sample preparation procedures suitable for both rotor-based (a.k.a. closed vessel)

Milestone ETHOS UP

The ETHOS UP with MAXI-24 HP high throughput rotor is designed to process increased volumes of sample types and weights within a single rotor-based platform. The MAXI-24 HP can process as many as 24 food samples simultaneously and complete the digestion in less than an hour. The digestion process is always under control via the built-in easyTEMP sensor that measures the temperature in all vessels and secures full control of the reaction. The ETHOS UP with MAXI-24 HP is an optimum tool to address the sample preparation of food samples and enable the operator to focus on what counts: increased throughput, high-quality data, and more profitable runs.

Milestone ultraWAVE 3

The ultraWAVE 3 is the hird generation of the digestion system based on the Milestone's patented Single Reaction Chamber (SRC) technology which uses a stainless-steel chamber with a PTFE vessel and cover. This approach revolutionized sample preparation offering superior performance, higher productivity, and unmatchable ease of use. The SRC technology, enables streamlining of the sample preparation workflow, removing the time-consuming handling typically involved in this process. At the same time, ultraWAVE 3 offers superior performance in terms of temperature and pressure capabilities, which in turn lead to the ability to digest higher sample masses with less acid, producing superior digestion and analysis quality. The ultraWAVE 3 combines productivity, ease of use, and better workflow into a single platform without compromising the performance.

microwave systems and for Single Reaction Chamber (SRC) (a.k.a. autoclave style) microwave systems. The FDA is currently discussing how to improve this method, not only to meet the lower heavy metal limits in the 2021 Baby Food Safety Act with greater confidence, but to ensure that the even lower limits expected in the future can also be met. Of course, measuring such low levels of trace elements requires the analyst to work in a clean environment with rugged procedures to control the contaminations and, in the previous section, we provided some solutions to make that easier. Another area on which FDA is focusing is reducing sample dilution factors to lower the method LOD/LOQ. As such, there is interest in how the latest developments in microwave sample preparation can contribute to attaining the ambitious measurement targets. A comprehensive recovery study was therefore performed with the aim of evaluating the ability of digestion procedures developed with the ultraWAVE 3 and ETHOS UP platforms to reduce method dilution factors for baby food analysis. Success in this endeavor is dependent on the ability of the digestion systems to process larger sample masses using limited acid volumes without affecting the quality of the digestion. High digestion quality is defined for this study as full analyte recovery with minimal matrix-origin interferences (e.g., as there could also be matrix-origin interferences due to high acid or dissolved solids concentrations, low residual carbon content). For this study, a mixed batch of food/infant samples, both dry and wet (dry-mass equivalent limit ≤ 0.5 g), were used. As shown in *Table 4*, the samples consisted of two reference materials (JRC-ERM and FAPAS), one quality control material (FAPAS), and four commercial samples. The commercial samples were spiked prior to digestion with a standard solution containing As, Cd, Hg, and Pb. The spike level addition was calculated to obtain a spike concentration for each element of 1 µg/L in the final analytical solution. The analysis of the samples was performed with a triple-quadrupole

Table 4. Food/infant food samples, both dry and wet, were used.

Sample type	*Sample ID*	*Description*
Certified Reference Material	ERM BD-150	Skimmed milk powder (trace elements)
Certified Reference Material	TFV002RM	Heavy metals in milk powder
QC material	T07413QC	Heavy metals in Infant Cereals (rice based)
Commercial	BF-MEAT	Baby food – Meat
Commercial	BF-VEG	Baby food – Vegetables
Commercial	BF-FISH	Baby food – Fish
Commercial	FJ	Fruit juice

ICP-MS system using the operating conditions listed in *Appendix*. For both ultraWAVE 3 and ETHOS UP, the data related to the new procedures described below are compared with the data for the same samples prepared using the standard EAM 4.7 method.

3.1 ultraWAVE 3 "closer to zero" plan procedure

Among the available technologies, SRC deserves particular attention (*Figure 6*). In SRC, the reaction chamber is hermetically closed and pressurized with an inert gas so that the vessels can reach temperatures as high as 300 °C and pressures as high as 199 bar. As an additional advantage, SRC uses simple and inexpensive vial-type digestion containers, along with different types of racks where, with the latest ultraWAVE 3 release, very high sample throughput can be achieved. As just mentioned, the key point of the SRC technology is the capability to withstand extremely high temperatures and pressures. The direct benefit coming from this is the ability to fully digest larger sample masses with lower acid volumes, when compared to rotor-based systems. *Table 5* lists the dilution factors achievable with the standard EAM 4.7 autoclave style microwave method along with the optimized methods tested in this work. In this study, hydrogen peroxide was not used for the new proposed methods. H_2O_2 is generally used to enhance the oxidizing power of the digestion mixture, moreover, it also helps to re-generate HNO_3 from NO reaction gas leading to more acid availability and lower pressure in the system. ultraWAVE 3, thanks to its high pressure and temperature capability, does not require H_2O_2 to get complete digestion, thus lowering reagent consumption and facilitating better control of the analytical blank. All the mentioned methods were tested on different samples and the recovery of As, Cd, Pb, and Hg are reported in the following tables. The acceptance criteria were 80-120%, the same as reported for EAM 4.7. In *Tables 6 and 7* the recovery data working with the EAM 4.7 method (method details in *Table 5*) are reported.

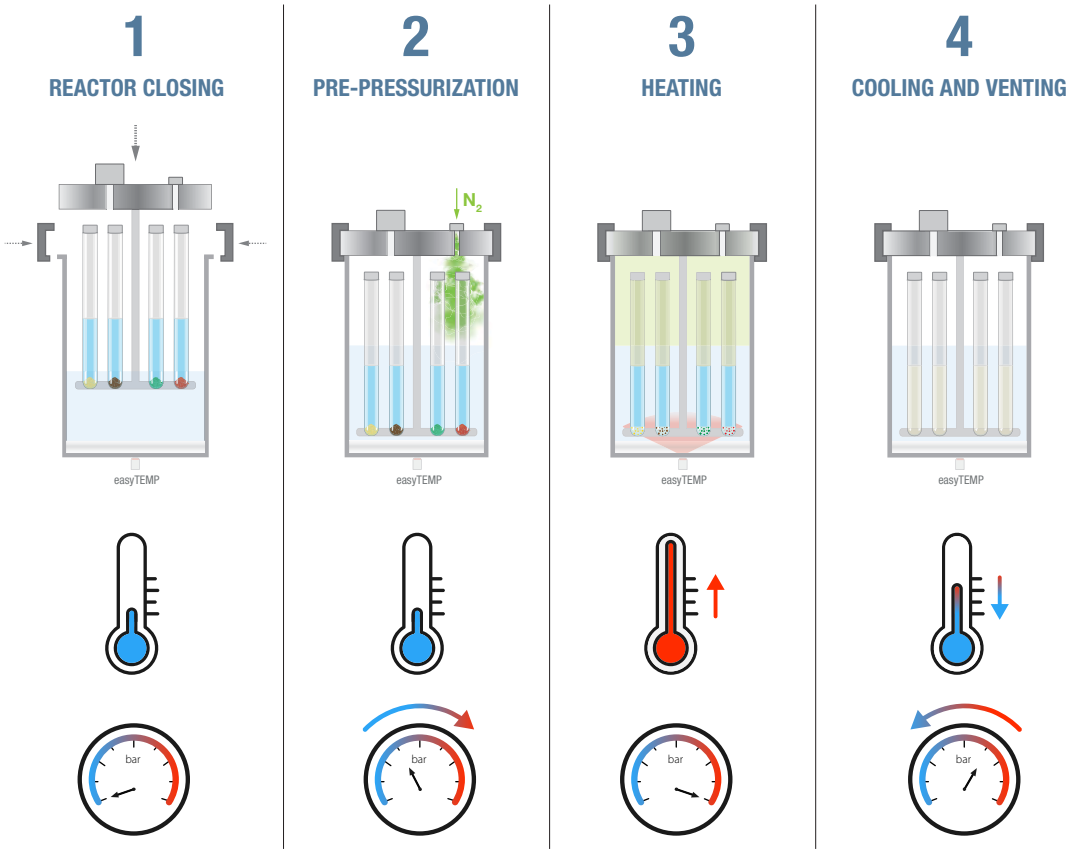

Figure 6. SRC Operating sequence

Table 5. ultraWAVE 3 methods for baby food digestion and their dilution factors.

Sample type	*Method*	*Sample amount*	*Digestion mixture*	*Final volume (mL)*	*Final Acidity* (V/V)*	*DIL FACT*
1	EAM 4.7	0.5 g (dry samples)	5 mL HNO_3 + 1 mL H_2O_2 (+ 0.25 mL HCl after digestion)	50	5% HNO_3 0.5% HCl	x 100
2	Lower acid volume	0.5 g (dry samples)	2.5 mL HNO_3 + 0.25 mL HCl	25	5% HNO_3 0.5% HCl	x 50
3	Larger sample mass	1 g (dry samples)	5 mL HNO_3 + 0.25 mL HCl	50	5% HNO_3 0.5% HCl	x 50
4	Larger sample mass/ Lower dilution	1 g (dry samples)	5 mL HNO_3 + 0.25 mL HCl	25	10% HNO_3 1% HCl	x 25
5	EAM 4.7	5 g (wet samples)	5 mL HNO_3 + 1 mL H_2O_2 (+ 0.25 mL HCl after digestion)	50	10% HNO_3 0.5% HCl	x 10
6	Lower acid volume	5 g (wet samples)	2.5 mL HNO_3 + 0.25 mL HCl	25	5% HNO_3 0.5% HCl	x 5

**Considering 10% as maximum acidity tolerable for ICP-MS, final acidity was estimated considering 50% oxidative acid consumption as reported in the EAM 4.7 method. Note that for methods 2-4 and 6 the listed final acidity exceeds the real value due to a lower sample to acid ratio. In this study, a conservative approach was applied for the calculation using the same 50% consumption factor reported in EAM 4.7.*
*** Addition of 1 mL of DI water is recommended to minimize the exothermic reaction.*

Table 6. ultraWAVE 3 recovery study of As, Cd, Pb, and Hg in dry food samples applying EAM 4.7 method

ultraWAVE 3 – EAM 4.7 method – Dry samples				
		ERM BD-150	***TFV002RM***	***T07413QC***
	Sample weight (g)	0.5	0.5	0.5
	Final Vol.(mL)	50	50	50
As	Exp.Conc.(µg/kg)	NA	72.9	113
	Meas.conc. (µg/kg)	3.14	67.8	115
	Rec%	-	93	102
	RSD% (n=3)	5.9	3.0	1.3
Cd	Exp.Conc.(µg/kg)	11.4	21.2	32.8
	Meas.conc. (µg/kg)	10.2	21.3	33.0
	Rec%	89	101	101
	RSD% (n=3)	0.49	3.0	1.4
Hg	Exp.Conc.(µg/kg)	60	39.6	29.6
	Meas.conc. (µg/kg)	51.9	41.7	29.8
	Rec%	86	105	101
	RSD% (n=3)	0.97	1.9	0.59
Pb	Exp.Conc.(µg/kg)	19	52.9	44.9
	Meas.conc. (µg/kg)	14.3	47.1	44.9
	Rec%	81	89	100
	RSD% (n=3)	3.3	2.7	0.65

Table 7. ultraWAVE 3 recovery study of As, Cd, Pb, and Hg in wet food samples applying EAM 4.7 method.

ultraWAVE 3 – EAM 4.7 method – Wet samples					
		BF-MEAT	***BF-VEG***	***BF-FISH***	***FJ***
	Sample weight (g)	5	5	5	5
	Final Vol. (mL)	50	50	50	50
As	Measured conc. (µg/kg)	1.82	2.19	20.8	1.11
	Spike Rec%	107	94	103	97
	RSD% (n=3)	6.2	3.3	8.5	4.5
Cd	Measured conc. (µg/kg)	7.37	9.9	6.02	1.01
	Spike Rec%	104	80	88	92
	RSD% (n=3)	7.3	1.8	8.0	0.15
Hg	Measured conc. (µg/kg)	0.48	<	0.97	<
	Spike Rec%	80	86	87	90
	RSD% (n=3)	8.2	8.3	3.9	3.9
Pb	Measured conc. (µg/kg)	2.64	12.6	2.51	0.75
	Spike Rec%	103	95	103	112
	RSD% (n=3)	6.6	5.5	3.0	2.9

In *Tables 8 and 9* the data obtained decreasing by half the volume of the acid used in EAM 4.7 method are reported. In the following last two recovery studies related to ultraWAVE 3 a new procedure was evaluated where double the sample mass (method details in *Table 5*) was used compared to EAM 4.7 method.

Table 8. ultraWAVE 3 recovery study of As, Cd, Pb, and Hg in dry food samples working with lower acid volume compared to EAM 4.7 method.

ultraWAVE 3 - Lower acid volume method – Dry samples				
		ERM BD-150	***TFV002RM***	***T07413QC***
	Sample weight (g)	0.5	0.5	0.5
	Final Vol. (mL)	25	25	25
As	Exp.Conc.(µg/kg)	NA	72.9	113
	Meas.conc. (µg/kg)	3.59	73.4	122
	Rec%	-	101	108
	RSD% (n=3)	11	4.4	4.4
Cd	Exp.Conc.(µg/kg)	11.4	21.2	32.8
	Meas.conc. (µg/kg)	10.0	21.7	34.4
	Rec%	88	102	105
	RSD% (n=3)	2.4	0.36	4.4
Hg	Exp.Conc.(µg/kg)	60	39.6	29.6
	Meas.conc. (µg/kg)	54.7	42.9	30.8
	Rec%	91	108	104
	RSD% (n=3)	1.3	1.6	1.5
Pb	Exp.Conc.(µg/kg)	19	52.9	44.9
	Meas.conc. (µg/kg)	15.8	50.6	42.8
	Rec%	83	96	95
	RSD% (n=3)	3.6	3.4	1.1

Table 9. ultraWAVE 3 recovery study of As, Cd, Pb, and Hg in wet food samples working with lower acid volume compared to EAM 4.7 method.

ultraWAVE 3 - Lower acid volume method – Wet samples					
		BF-MEAT	***BF-VEG***	***BF-FISH***	***FJ***
	Sample weight (g)	5	5	5	5
	Final Vol. (mL)	25	25	25	25
As	Measured conc. (µg/kg)	3.36	3.27	33.8	1.26
	Spike Rec%	113	115	114	115
	RSD% (n=3)	4.6	1.9	4.1	6
Cd	Measured conc. (µg/kg)	6.15	10.0	5.44	0.98
	Spike Rec%	100	85	95	89
	RSD% (n=3)	4.5	4.4	5.6	4.9
Hg	Measured conc. (µg/kg)	0	0.33	1.85	0.71
	Spike Rec%	99	92	87	88
	RSD% (n=3)	6.5	4.9	3.8	4.7
Pb	Measured conc. (µg/kg)	3.04	12.2	2.54	0.71
	Spike Rec%	97	87	94	95
	RSD% (n=3)	9.4	5.0	2.8	3.9

Thanks to the complete digestion achieved with this ultraWAVE 3 procedure, a lower dilution factor was applicable (*Tables 10 and 11*). The recovery data reported shows that the proposed methods enable lowering the dilution factor from x100 to x25 for dry samples and from x10 to x5 for wet samples by increasing the dry sample mass compared to what is typically digested with the EAM 4.7 method and decreasing the final sample dilution for both types of samples by half. Achieving a two-fold fold increase in dry sample mass compared to the EAM 4.7 method is possible thanks to the specific digestion chamber design of ultraWAVE 3, where the digestion takes place in a small vial that allows for optimization of the ratio of sample mass to acid volume. This, combined with the high performance and homogeneity of the heating reached with ultraWAVE 3, enables the development of these highly efficient methods.

Table 10. ultraWAVE 3 recovery study of As, Cd, Pb, and Hg in dry food samples working with larger sample mass and lower dilution factor compared to EAM 4.7 method.

ultraWAVE 3 – Larger sample mass/Lower dilution method - Dry samples				
		ERM BD-150	***TFV002RM***	***T07413QC***
	Sample weight (g)	1	1	1
	Final Vol. (mL)	25	25	25
As	Exp.Conc.(µg/kg)	NA	72.9	113
	Meas.conc. (µg/kg)	3.65	88.0	136
	Rec%	-	120	121
	RSD% (n=3)	2.9	3.7	4.0
Cd	Exp.Conc.(µg/kg)	11.4	21.2	32.8
	Meas.conc. (µg/kg)	11.8	21.0	36.0
	Rec%	104	102	109
	RSD% (n=3)	0.42	5.4	4.8
Hg	Exp.Conc.(µg/kg)	60	39.6	29.6
	Meas.conc. (µg/kg)	51.9	42.0	33.0
	Rec%	86	104	110
	RSD% (n=3)	6.1	6.0	3.0
Pb	Exp.Conc.(µg/kg)	19	52.9	44.9
	Meas.conc. (µg/kg)	18.3	46.0	43.0
	Rec%	96	87	94
	RSD% (n=3)	5.3	4.4	2.7

Table 11. ultraWAVE 3 recovery study of As, Cd, Pb, and Hg in wet food samples working with larger sample mass and lower dilution factor compared to EAM 4.7 method.

ultraWAVE 3 – Larger sample mass/Lower dilution method – Wet samples					
		BF-MEAT	***BF-VEG***	***BF-FISH***	***FJ***
	Sample weight (g)	5	5	5	5
	Final Vol. (mL)	100	100	100	100
As	Measured conc. (µg/kg)	4.07	0.18	81.4	2.88
	Spike Rec%	120	116	119	113
	RSD% (n=3)	4.4	2.8	1.5	2.6
Cd	Measured conc. (µg/kg)	0.08	8.43	6.82	0.08
	Spike Rec%	96	96	94	92
	RSD% (n=3)	1.8	1.2	0.81	2.5
Hg	Measured conc. (µg/kg)	0.14	0.15	3.29	0.39
	Spike Rec%	93	91	89	89
	RSD% (n=3)	6.5	6.3	8.7	2.3
Pb	Measured conc. (µg/kg)	0.4	1.15	0.91	0.5
	Spike Rec%	88	85	84	86
	RSD% (n=3)	0.96	2.0	0.42	1.6

3.2 **ETHOS UP "closer to zero" plan procedure**

A similar study was also performed for a rotor-based microwave system. Referring to the EAM 4.7 closed vessel microwave method and related dilution factor, an ETHOS UP system equipped with a MAXI-24 HP rotor was tested with new methodologies aiming to reduce dilution factors. The conditions used are reported in *Table 12*.
All the methods above were tested on different samples and the recoveries of As, Cd, Pb, and Hg are reported in the following tables.
In *Tables 13 and 14* the recovery data working with the EAM 4.7 method (method details in *Table 12*) are reported. In *Tables 15 and 16* the data obtained from decreasing by half the volume of the acid used in EAM 4.7 method are reported. Despite the clear different specifications between the rotor-based system and ultraWAVE 3 technology, the data reported on ETHOS UP recovery study demonstrated that MAXI-24 HP is suitable for this application field since it enables lowering the dilution factor from x200 to x100 for dry samples and from x20 to x10 for wet samples.

Table 12. ETHOS UP methods for baby food digestion and their dilution factors.

Sample type	*Method*	*Sample amount*	*Digestion mixture*	*Final volume (mL)*	*Final Acidity* (V/V)*	*DIL FACT*
7	EAM 4.7	0.5 g (dry samples)	8 mL HNO_3 + 1 mL H_2O_2 (+ 0.5 mL HCl after digestion)	100	8% HNO_3 0.5% HCl	x 200
8	EAM 4.7	5 g (dry samples)	8 mL HNO_3 + 1 mL H_2O_2 (+ 0.5 mL HCl after digestion)	100	8% HNO_3 0.5% HCl	x 20
9	Lower acid volume	0.5 g (dry samples)	4 mL HNO_3 + 0.5 H_2O_2 (+ 0.25 mL HCl)	50	8% HNO_3 0.5% HCl	x 100
10	Lower acid volume	5 g (dry samples)	4 mL HNO_3 + 0.5 H_2O_2 (+ 0.25 mL HCl)	50	8% HNO_3 0.5% HCl	x 10

Table 13. ETHOS UP recovery study of As, Cd, Pb, and Hg in dry food samples applying EAM 4.7 method.

ETHOS UP, MAXI-24 HP– EAM 4.7 method – Dry samples				
		ERM BD-150	TFV002RM	T07413QC
	Sample weight (g)	0.5	0.5	0.5
	Final Vol. (mL)	100	100	100
As	Exp.Conc.(µg/kg)	NA	72.9	113
	Meas.conc. (µg/kg)	2.7	74.2	128
	Rec%	-	102	114
	RSD% (n=3)	5.1	2.6	1.4
Cd	Exp.Conc.(µg/kg)	11.4	21.2	32.8
	Meas.conc. (µg/kg)	11.4	19.9	31.2
	Rec%	100	94	95
	RSD% (n=3)	6.3	1.8	7.0
Hg	Exp.Conc.(µg/kg)	60	39.6	29.6
	Meas.conc. (µg/kg)	54.5	40.8	28.8
	Rec%	91	103	97
	RSD% (n=3)	1.7	2.6	1.5
Pb	Exp.Conc.(µg/kg)	19	52.9	44.9
	Meas.conc. (µg/kg)	16.6	51.8	44.3
	Rec%	88	98	99
	RSD% (n=3)	1.3	4.6	1.8

Table 14. ETHOS UP recovery study of As, Cd, Pb, and Hg in wet food samples applying EAM 4.7 method.

ETHOS UP, MAXI-24 HP– EAM 4.7 method – Wet samples					
		BF-MEAT	BF-VEG	BF-FISH	FJ
	Sample weight (g)	5	5	5	5
	Final Vol. (mL)	100	100	100	100
As	Measured conc. (µg/kg)	4.07	0.18	81.4	2.88
	Spike Rec%	120	116	119	113
	RSD% (n=3)	4.4	2.8	1.5	2.6
Cd	Measured conc. (µg/kg)	0.08	8.43	6.82	0.08
	Spike Rec%	96	96	94	92
	RSD% (n=3)	1.8	1.2	0.81	2.5
Hg	Measured conc. (µg/kg)	0.14	0.15	3.29	0.39
	Spike Rec%	93	91	89	89
	RSD% (n=3)	6.5	6.3	8.7	2.3
Pb	Measured conc. (µg/kg)	0.4	1.15	0.91	0.5
	Spike Rec%	88	85	84	86
	RSD% (n=3)	0.96	2.0	0.42	1.6

Table 15. ETHOS UP recovery study of As, Cd, Pb, and Hg in dry food samples working with lower acid volume compared to EAM 4.7 method.

ETHOS UP, MAXI 24 HP– Lower acid volume method – Dry samples				
		ERM BD-150	TFV002RM	T07413QC
	Sample weight (g)	0.5	0.5	0.5
	Final Vol. (mL)	50	50	50
As	Exp.Conc.(µg/kg)	NA	72.9	113
	Meas.conc. (µg/kg)	4.22	80.8	131
	Rec%	-	111	116
	RSD% (n=3)	7.2	5.6	1.1
Cd	Exp.Conc.(µg/kg)	11.4	21.2	32.8
	Meas.conc. (µg/kg)	12.0	22.5	32.2
	Rec%	105	106	98
	RSD% (n=3)	5.9	3.3	5.9
Hg	Exp.Conc.(µg/kg)	60	39.6	29.6
	Meas.conc. (µg/kg)	54.4	39.9	26.7
	Rec%	91	101	90
	RSD% (n=3)	4.7	4.9	4.1
Pb	Exp.Conc.(µg/kg)	19	52.9	44.9
	Meas.conc. (µg/kg)	18.2	51.3	42.2
	Rec%	96	97	94
	RSD% (n=3)	8.7	0.74	1.8

Table 16. ETHOS UP recovery study of As, Cd, Pb, and Hg in wet food samples working with lower acid volume compared to EAM 4.7 method.

ETHOS UP, MAXI 24 HP– Lower acid volume method – Wet samples					
		BF-MEAT	BF-VEG	BF-FISH	FJ
	Sample weight (g)	5	5	5	5
	Final Vol. (mL)	25	25	25	25
As	Measured conc. (µg/kg)	4.37	0.48	81.4	3.18
	Spike Rec%	115	110	117	117
	RSD% (n=3)	7.4	5.9	1.1	0.84
Cd	Measured conc. (µg/kg)	9.24	0.72	7.38	0.08
	Spike Rec%	85	81	81	83
	RSD% (n=3)	9.7	5.5	6.8	5.6
Hg	Measured conc. (µg/kg)	0.08	0.06	2.72	0.06
	Spike Rec%	93	85	85	85
	RSD% (n=3)	4.0	2.4	3.3	5.1
Pb	Measured conc. (µg/kg)	3.04	12.2	2.54	0.71
	Spike Rec%	80	87	82	89
	RSD% (n=3)	7.3	4.7	3.8	4.6

3.3 Final considerations on sample preparation methodologies

The data reported demonstrate the suitability of the ultraWAVE 3 and ETHOS UP systems for the EAM 4.7 method. In particular, they show how ultraWAVE 3's higher performance is a good match for the Baby Food Safety Act 2021 and its upcoming more demanding requirements.
For dry baby food samples, applying an ultraWAVE 3 procedure with larger sample mass and lower dilution factor can reduce the method LOQ's by a factor of four, which in this study would lower it from 0.9 to 0.22 µg/kg for As, Cd, and Pb, and from 0.45 to 0.12 µg/kg for Hg.
For wet baby food samples, the method LOQ's can be reduced by a factor of two, which for this study would lower it from 0.09 to 0.045 µg/kg for As, Cd, Pb, and from 0.045 to 0.023 µg/kg for Hg. While the LOQ's measured in this study using the EAM 4.7 procedure are comfortably below the action limits proposed in the ***"The Baby Food Safety Act of 2021"***, when those action limits are reduced in the future, as is required by law, the level of confidence in the analysis will be reduced. According to one industry expert, "If the action limits were reduced by a factor of 2 to 5 times in the future, ICP-MS would probably struggle to meet the required LOQ's, unless the sample weight could be increased and/or the dilution factor lowered." [12] As the FDA has proposed investigating, this study demonstrates that baby food digestion methods using increased sample mass and lower final dilution, when coupled with systems capable of completely digesting samples under those conditions, can indeed lower the LOQ's such that heavy metals in baby foods can be determined, both now and in the future, with the appropriate levels of confidence.

Milestone DMA-80 evo

Do you know about Direct Mercury Analysis? Ask analysts about their ideal analysis technique and they probably will describe analytical measurements performed directly on untreated samples, without any sample preparation steps, and with a simple strategy for analytical calibration. Something like: insert samples, wait a few minutes, and get results! To many analysts, this may seem like a dream. But fortunately, technology exists today that fits this description. Milestone has developed a direct mercury analyser, DMA-80 *evo*, capable of directly determining mercury in nearly any kind of sample in about 5 min.

Table 17. Hg from results from baby food analyzed with DMA-80 evo.

		BD-150	*TFV002RM*	*T07413QC*	*BF-MEAT*	*BF-VEG*	*BF-FISH*	*FJ*
Hg	Sample weight (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Exp.Conc.(µg/kg)	60	39.6	29.6	25	25	25	25
	Exp.Mass.(ng Hg)	12	7.92	5.92	5*	5*	5*	5*
	Meas.Mass.(ng Hg)	11.66	7.97	5.79	4.77	4.83	5.12	4.91
	Rec%	97	101	98	95	97	102	98
	RSD% (n=3)	0.97	1.2	0.59	0.88	1.0	1.1	1.1

**Commercial samples were spiked with 100 µL of a 50 µg/L std solution (5 ng Hg) directly into the DMA-80 sample boats.*

Table 18. Typical LOQ calculation for DMA-80 Hg determination.

DMA-80 evo TriCell		*Typical ASQL (ng)*	*Sample mass (g)*	*Dil. factor*	*LOQ (µg/kg)*	*Safety Act Action Limit (µg/kg)*
Dry/Wet samples	Hg	0.03	0.2	1	0.15	2

4.

Concluding remarks: an integrated, rugged, and greener sample preparation workflow for trace metal analysis

As you know and as we have discussed in the previous sections, trace elemental analysis depends on modern instrumentation with extreme sensitivity, but the analytical capability of these instruments will not be effective without optimized analytical procedures and trained analysts.
The analyst culture must be adapted to think about contamination and how to control the analytical blank. It is well known that trustworthy results could not be obtained without proper sample preparation and without proper control of the analytical blank. We propose here an integrated and complete workflow, specifically developed for food testing labs, to be rugged, easy to apply, and have less impact on the environment (*Figure 7*).

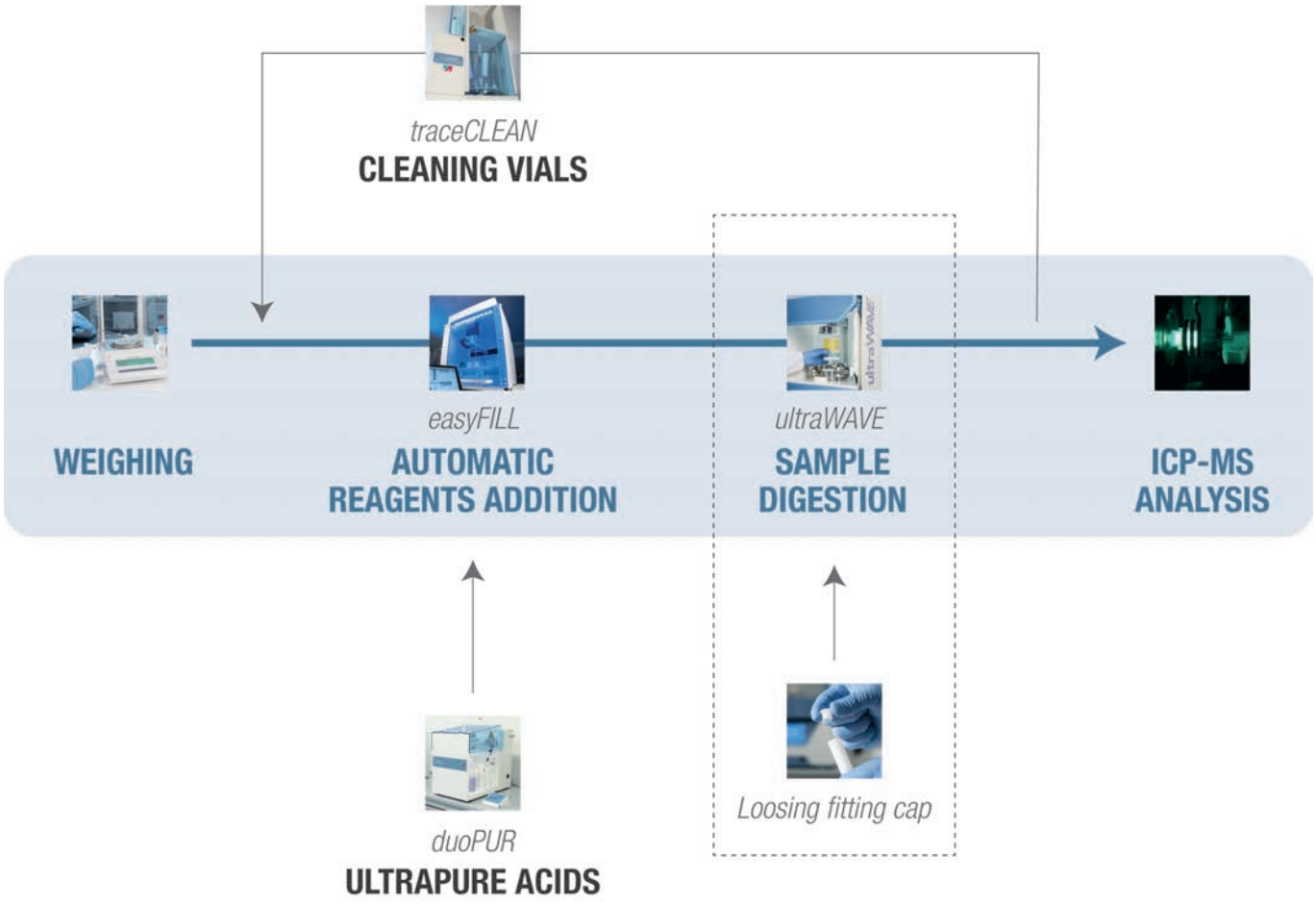

Figure 7. Sample preparation workflow for trace elemental determination

In this workflow, manual sample manipulation is limited as much as possible:

- duoPUR: starting from reagent-grade acid, produces fresh, ultra-pure acid on demand
- easyFILL: automatically adds all the required acids to the digestion vials
- ultraWAVE 3: enables digestion of up to 20 samples per run working with high sample mass and low acid volume for superior LOQ's
- traceCLEAN: automated and robust vial cleaning guaranteed by continuously generated fresh acid vapors

The proposed workflow enables achievement of accurate trace element results, and it is pivotal for meeting the stringent requirements for determining As, Cd, Pb, and Hg in baby foods. However, despite being essential, it is not enough to think only about blank control and analysis accuracy. Nowadays, analytical procedures must also be friendly to the environment. To develop a greener sample preparation workflow, we should also consider the following analysis-related areas that have an impact on the environment and can lead to a "green chemistry" approach [9]:

1. Production of reagents: raw material, energy, time, purity, subproducts, yield, and generated waste.
2. Chemical and physical properties of reagents: boiling point, flammability, corrosiveness, stability, shelf life, ease of recovery, and ease of handling.
3. Lifetime of equipment, vessels, and sensors.
4. Sample amount needed.
5. Mass, volume, and concentration of reagents.
6. Need of gases or special reagents.
7. Energy needed for promoting and keeping the reaction system heated – use of energy-effective apparatus.
8. Reaction conditions: temperature, pressure, and time.
9. Generation of excessively reactive or dangerous products.
10. Volume and toxicity of gases generated.
11. Risk to the analyst and to the environment.
12. Volume of wastes generated.
13. Recycle of reagents.

The proposed microwave-assisted sample preparation procedures for trace analysis of baby foods were developed with consideration for these principles. Consequently, high masses of baby food samples can be efficiently digested using less acid nitric solution and all steps, i.e., from weighing to measurement, can be performed with reduced sample manipulation by the analyst, avoiding losses and contamination. The use of limited volumes of nitric acid solutions, prepared from reagent-grade acids purified by sub-boiling distillation, lead to better blanks, lower cost of reagents, and less waste generation. Clean acid are produced on demand and only of the amounts required, so there is no risk of needing to dispose of excess ultrapure reagents that can become contaminated over time. Furthermore, we avoided the use of hydrogen peroxide or any other concentrated reagent and consequently better digestion blanks were obtained. The use of traceCLEAN ensures proper decontamination of digestion vessels by using and re-using a limited amount of acid. The developed procedures combine blank strategies for proper control of analytical blanks that meet the needs for trace elemental analysis of baby foods, with green strategies for decreasing impacts on analysts, laboratory staff, and the environment. In *Table 17* are reported data from Hg analyses performed with DMA-80 *evo* on the same CRM materials used in this study. In addition to the excellent recoveries, *Table 18* demonstrates how the DMA-80 *evo* method achieved an LOQ suitable for the Baby Food Safety Act Hg action limit and even has a performance margin for when the action limit becomes lower in the future. Given the several challenges of determining Hg by ICP-MS, more and more laboratories are turning to Direct Mercury Analysis for their low-level mercury determinations, such as those described in this baby food analysis study.

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Appendix

PROCEDURES

Acid purification - duoPUR

HNO_3: 45% power ≈ 60 mL/h (4-h cycle)
HCl: 35% power ≈ 50 mL/h (4-h cycle)

Reactor cleaning – traceCLEAN

Total time	01:30:00
Temp.	240 °C
Acid volume	500 mL

ultraWAVE 3 digestion procedure

- Pre-loaded pressure (N_2): 40 bar
- Cooling temperature (liquid chiller): 8 °C
- Vessel cooling activated beyond 40 °C
- Pressure release below 80 °C
- Pressure release rate: 8 bar/min

MW program:

	Time	*Temp (T1)*	*Temp (T2)*	*P*	*Power*
1	00:20:00	250 °C	60 °C	130 bar	1500 W
2	00:15:00	250 °C	60 °C	130 bar	1500 W

ETHOS UP, MAXI-24 HP digestion procedure

MW program:

	Time	*Temp*	*Power*	*Fan*
1	00:20:00	210 °C	1800 W	***
2	00:15:00	210 °C	1800 W	***
3	00:20:00	Cooling		***

Quantification

Analytical instrumentation: Triple-quadrupole ICP-MS
Instrumental parameters:

Parameters	***Settings***
RF Plasma power	1.55 kW
Cones	Pt cones
Nebulizer Gas flow rate	1.05 L/min
Auxiliary Gas flow rate	0.8 L/min
Cool Flow	14 L/min
Insert	High matrix
Sampling depth	8 mm
Pump rate	15 rpm
Autodilution sample loop	2 mL
CRC flow	SQ-KED: 4.85 mL/min
	TQ-O2: 0.34 mL/min
Integration time	As and Hg 200 ms; Cd 100 ms; Pb 50 ms
Replicates	3
Internal standards	45Sc 100 ppb
	73Ge 100 ppb
	103Rh 10 ppb
	193Ir 10 ppb
Internal standard calculation	Interpolation

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