Application note | 003160

Trace elemental analysis

Trace metals analysis in baby food using inductively coupled plasma mass spectrometry (ICP-MS)

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Keywords

Baby food, AGD, inductively coupled plasma mass spectrometry, toxic elements, interference suppression

Goal

This application note will highlight the complete and comprehensive workflow for toxic elements analysis in various baby foods. An automatized and reproducible acid digestion sample preparation combined with a sensitive and robust method based on triple quadrupole ICP-MS allows a wide range of trace elements present in the samples to be quantified without any compromise in respect to applicable regulations.

Introduction

Many countries have established their own regulations and guidelines to ensure the safety and quality of baby food products. These regulations are generally based on scientific research and risk assessments conducted by health and food safety authorities. Among a range of other contaminants, toxic heavy metals, such as lead, arsenic, cadmium, and mercury, are subject to screening. Infants and young children up to the age of three represent an especially vulnerable group susceptible to different illnesses and potential lifelong neurological damage through exposure to toxic heavy metals.

The Baby Food Safety Act of 2021 requires manufacturers and the FDA to take long overdue action by setting maximum levels of inorganic arsenic (10 $\mu g \cdot kg^{-1}$, 15 $\mu g \cdot kg^{-1}$ for cereal), lead (5 $\mu g \cdot kg^{-1}$, 10 $\mu g \cdot kg^{-1}$ for cereal), cadmium (5 $\mu g \cdot kg^{-1}$, 10 $\mu g \cdot kg^{-1}$ for cereal), and mercury (2 $\mu g \cdot kg^{-1}$) allowed in baby food.

Since January 2022, baby food manufacturers need to meet these maximum levels for toxic elements by testing their final products (because only ingredient testing significantly underestimates toxic heavy metal levels in the final product), and to post the results of their product testing online twice per year. Similarly, as the FDA is working on a "closer to zero plan", the European Union has established regulations and maximum limits for various contaminants, including toxic elements, in baby food.

Moreover, essential minerals like iron, zinc, copper, calcium, and magnesium are important for the healthy growth and development of infants. Monitoring their levels ensures that the baby food provides adequate nutrition. Depending on the specific formulation and ingredients of the baby food, additional trace elements such as selenium, manganese, chromium, and molybdenum may also be analyzed to assess their levels and potential impact on infant health.

ICP-MS (inductively coupled plasma mass spectrometry) is the commonly used analytical technique to measure trace elements in various samples, including baby food. The specific trace elements to be checked by ICP-MS in baby food may vary depending on the purpose of the analysis and the specific concerns related to the safety and quality of the product.

Table 1. Instrument parameters of the iCAP MTX ICP-MS

Parameter	Value
Nebulizer	iCAP MX Series Nebulizer
Interface cones	Ni – tipped sample and skimmer
Spray chamber	Cyclonic quartz
Injector	Quartz, 1.5 mm ID
Torch	Quartz
Auxiliary flow (L·min^{-1})	0.8
Cool gas flow (L·min^{-1})	14
Automatic dilution	Level-5
AGD humidifier	ON
Nebulizer flow (L·min^{-1})	0.45
Argon gas dilution flow (L·min^{-1})	0.50
QCell KED flow (mL. min^{-1})	4.91
QCell O_2 flow (mL. min^{-1})	0.31
RF power (W)	1,550
Sampling depth (mm)	8
Number of replicates	3
Spray chamber temp. (°C)	2.7

In this study, toxic elements but also essential minerals will be determined to give an overview of elements present in a variety of baby food products.

Experimental

A Thermo Scientific™ iCAP™ MTX ICP-MS and a Thermo Scientific™ iSC-65 Autosampler were used for analysis. The ICP-MS was operated using the parameters highlighted in Table 1. To facilitate the robustness to analyze a high number of potentially very different sample types, the instrument was operated using argon gas dilution (AGD), applying a mild (approximately 5 times) dilution to all samples. This ensures the productivity and uptime requirements in a high productivity laboratory environment can be met. To meet the stringent detection limit requirements put in place by regulatory authorities, the iCAP MTX ICP-MS was operated in SQ-KED and TQ-O_2 modes to confidently eliminate all polyatomic and isobaric interferences but also deliver the sensitivity needed to reach the requested quantification limits for the critical elements.

Sample and standard preparation

Six baby food products were selected for this study and were purchased in a local supermarket. Products with different compositions (i.e., content of fat, moisture content, or dry powders) were chosen to obtain a range of different matrices and analyte concentrations. Three certified reference materials were analyzed to check the method accuracy and precision. All the samples are described in Table 2.

Table 2. List of baby food samples investigated in this study

Label	Type	Comment	Sample preparation strategy
TFV002RM - Skimmed milk powder	CRM	Carbohydrates	Dry
TM07413 - Infant cereal (rice based)	CRM	Carbohydrates	Dry
LGC7103 - Sweet digestive biscuit	CRM	Carbohydrates, fat	Dry
Yogurt	Sample	Carbohydrates, fat	Fat
Apple-nectarine-banana	Sample	Carbohydrates, mostly fruit sugar, dietary fiber	Wet
Carrot	Sample	Dietary fiber	Wet
Salmon	Sample	High fat and protein	Wet
Lamb	Sample	High fat and protein	Wet
Infant biscuit	Sample	Carbohydrates, fat	Dry

Despite the different characteristics, all samples were prepared using microwave assisted acid digestion to apply a single preparation method across all types of sample materials. An UltraWAVE microwave system (Milestone Srl., Sorisole, Bergamo, Italy) was used for the sample preparation. The UltraWAVE is a digestion system based on the patented Single Reaction Chamber (SRC) technology, which uses a stainless-steel chamber with a PTFE vessel and cover. The SRC technology enables streamlining of the sample preparation workflow and removes the time-consuming handling typically involved in this process. At the same time, the UltraWAVE system 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.

Due to the capacity of the UltraWAVE system to digest high sample masses, and the ability of the iCAP MTX ICP-MS to analyze samples with high amounts of dissolved solid without further dilution, 0.5 g of dry or fat samples and 1.0 g of wet samples were weighted in the Quartz UltraWAVE tubes. Digested samples were finally made up to a final volume of 50 mL with ultra-pure water and were directly placed on the autosampler for analysis. The sample preparation details are given in Table 3.

To improve the reproducibility and the productivity, a Milestone EasyFILL acid dispenser is used to automatically add 2 mL of HNO_3 and 0.5 mL of HCl in all UltraWAVE tubes. The EasyFILL utilization allows completion of the workflow and automatization of the sample preparation with minimum operator interaction.

During all analyses, an internal standard solution was automatically added (500 µg·L^{-1} Sc and Ge; 20 µg·L^{-1} Rh and Ir) before nebulization to compensate for any sample matrix effects in the plasma.

Calibration curves were prepared with a multi-element stock solution containing 34 elements. A low QC (0.2 µg·L^{-1} and 0.004 µg·L^{-1} for Hg) and a high QC (25 µg·L^{-1} and 0.5 µg·L^{-1}) were independently prepared to verify the validity of the calibration curve throughout the run (see Table 4 for details).

For the analysis of common nutrients such as Na, Mg, K, Ca, P, S, and Si, an additional calibration curve was prepared alongside an own QC check (1 mg·L^{-1}).

Results and discussion

Calibration

Table 5 summarizes the analytical figures of merit for all analytes including the correlation coefficients, the limits of quantification, and the quantification limits of the method (MLOQ). Whereas the limit of quantification (LOQ) only considers what can be quantified by the instrument, the method quantification limit (MLOQ) also accounts for the complete sample preparation, i.e., the sample weight and the dilution factor. Specifically highlighted in the table are the results obtained for As, Cd, Hg, and Pb, elements which are of specific regulatory concern due to their toxicity and risk for bioaccumulation. As can be seen, the achieved MLOQs are at least a factor two lower compared to the nominal limits specified in EAM 4.7.

Table 3. Sample preparation protocols and resulting dilution factors for dry and wet sample types

Sample type	Sample amount	Digestion mixture	Final volume (mL)	Final acidity (V/V)	Dilution factor
Dry and fat samples (milk powder, infant cereal, biscuit, yogurt)	0.5 g	2 mL HNO_3 + 0.5 mL HCl	50	4% HNO_3 1% HCl	× 100
Wet samples (meat, fish, vegetable, fruit)	1 g	2 mL HNO_3 + 0.5 mL HCl	50	4% HNO_3 1% HCl	× 50

Table 4. Calibration standard and QC for trace elements

Trace elements	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	QC Low	QC High
Multielement solution	0.1 µg·L^{-1}	0.5 µg·L^{-1}	1.0 µg·L^{-1}	10 µg·L^{-1}	20 µg·L^{-1}	50 µg·L^{-1}	100 µg·L^{-1}	0.2 µg·L^{-1}	25 µg·L^{-1}
Hg	0.002 µg·L^{-1}	0.01 µg·L^{-1}	0.02 µg·L^{-1}	0.2 µg·L^{-1}	0.4 µg·L^{-1}	1.0 µg·L^{-1}	-	0.004 µg·L^{-1}	0.5 µg·L^{-1}
Na. Mg. Si. P. S. K. Ca	0.02 mg·L^{-1}	0.1 mg·L^{-1}	0.5 mg·L^{-1}	2.0 mg·L^{-1}	10 mg·L^{-1}	50 mg·L^{-1}	-	1.0 mg·L^{-1}	N/A

Table 5. Analytical figures of merit for all analytes, including R^2, IDL, and MQL for dry and wet sample types. Values in bold indicate concentrations given in $mg \cdot kg^{-1}$. Highlighted are the calculated limits for toxic elements As, Cd, Hg, and Pb.

		EAM 4.7 measured limits iCAP MTX			Typical ICP-SQMS EAM 4.7 Nominal analytical limits
	R^2	LOQ ($\mu g \cdot kg^{-1}$)	MLOQ (dil 50) Wet baby food ($\mu g \cdot kg^{-1}$)	MLOQ (dil 100) Dry baby food ($\mu g \cdot kg^{-1}$)	LOQ ($\mu g \cdot kg^{-1}$)
^{11}B	0,9913	0.013	0.668	1.336	
^{23}Na	0.9999	0.001	0.072	0.145	
^{24}Mg	0.9999	0.001	0.043	0.086	
^{28}Si	0.9998	0.019	0.974	1.947	
^{27}Al	>0.9999	3.161	158.0	316.1	
^{28}Si	0.9998	0.019	0.974	1.947	
^{31}P	0.9999	0.002	0.079	0.159	
^{32}S	0.9999	0.004	0.203	0.405	
^{39}K	0.9999	0.026	1.325	2.651	
^{44}Ca	0.9999	0.013	0.625	1.250	
^{48}Ti	0.9999	0.023	1.147	2.295	
^{51}V	0.9999	0.091	4.550	9.101	
^{52}Cr	0.9998	0.107	5.390	10.78	48.9
^{55}Mn	0.9999	0.060	3.035	6.071	21.2
^{56}Fe	0.9999	0.153	7.679	15.36	
^{59}Co	0.9997	0.004	0.235	0.471	
^{60}Ni	0.9985	0.372	18.63	37.26	58
^{63}Cu	0.9995	0.093	4.690	9.381	54.7
^{66}Zn	0.9998	0.610	30.47	60.95	340
^{75}As	0.9999	0.054	2.718	5.436	11.6
^{80}Se	0.9999	0.084	4.204	8.408	66.1
^{85}Rb	0.9998	0.094	4.697	9.395	
^{88}Sr	0.9999	0.051	2.538	5.077	
^{98}Mo	0.9999	0.018	0.895	1.791	47.1
^{107}Ag	0.9999	0.008	0.378	0.758	
^{111}Cd	0.9999	0.008	0.412	0.824	3.71
^{118}Sn	0.9999	0.037	1.831	3.662	
^{121}Sb	0.9999	0.011	0.562	1.124	
^{125}Te	0.9999	0.042	2.109	4.218	
^{138}Ba	0.9999	0.042	2.116	4.233	
^{139}La	0.9999	0.002	0.085	0.170	
^{140}Ce	0.9999	0.003	0.136	0.272	
^{202}Hg	0.9995	0.007	0.355	0.712	7.82
^{205}Tl	0.9998	0.002	0.087	0.175	
^{208}Pb	0.9998	0.004	0.195	0.391	10.9
^{209}Bi	0.9999	0.004	0.214	0.428	
^{238}U	0.9997	0.004	0.189	0.379	

The calibration curve accuracy was monitored throughout the sequence by incorporating QC checks for all elements every 10 samples. Figure 1 represents the recoveries obtained for the QC check at a concentration of 0.2 µg·L^{-1} QC for all trace elements.

All QC recoveries were found to be within the ±20% range, hence demonstrating that the iCAP MTX ICP-MS instrument offers the sensitivity and stability required to routinely analyze baby food samples without any additional dilution before the analysis. This is specifically important for toxic elements such as arsenic, cadmium, mercury, and lead.

Robustness

The workflow was designed to analyze a wide variety of baby food products using a single method. Due to the variability of the sample composition, the instrument´s robustness to run all the different samples in one sequence was investigated thoroughly. This was accomplished by analyzing the different samples periodically within the same sequence. To check the internal standard (IS) response during a working day of analysis (8 hours) and over several days, work was carried out on four different days.

The IS recovery graph is automatically generated for ^{45}Sc, ^{73}Ge, ^{103}Rh, and ^{193}Ir in both modes SQ-KED and TQ-O_2 through Qtegra Software (Figure 2).

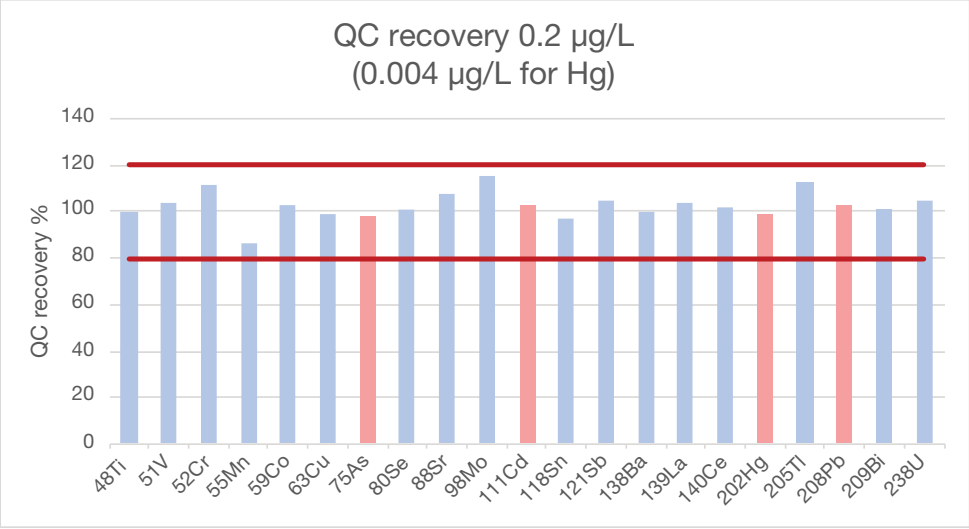

Figure 1. Recovery (%) of QC 0.2 µg/L for trace elements. Red highlights the recovery for As, Cd, Hg, and Pb.

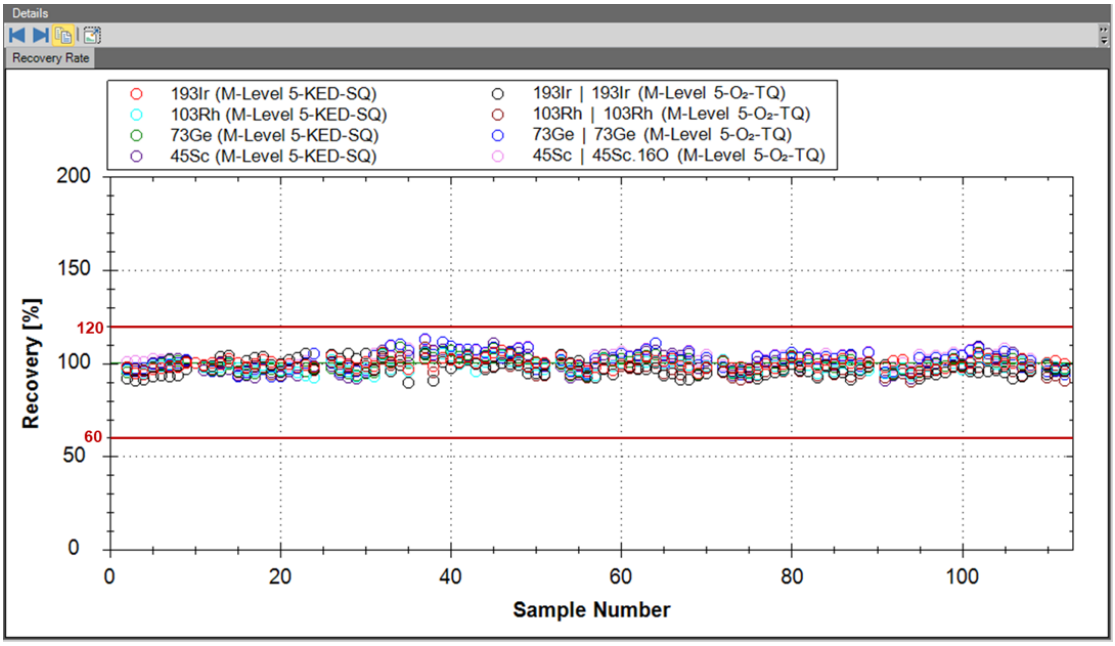

Figure 2. Internal standard stability (Sc, Ge, Rh, Ir) for 8-hour analysis of baby food samples

Thanks to the automatic 5-times dilution delivered by the iCAP MTX ICP-MS, the four internal standards (Sc, Ge, Rh, and Ir) could be read out with similar response (in the limit of 60–120% as recommended in the EMA 4.7 by the FDA) compared to the first sample in both modes utilized (KED and TQ-$O_{2)}$.

Accuracy

The workflow accuracy for determining the concentration level of toxic elements was checked by the analysis of three certified reference materials. Each CRM was prepared in triplicate to evaluate the reproducibility of the sample preparation and the analysis. All results are summarized in Table 6.

Optimized with the 5-fold dilution, the iCAP MTX ICP-MS instrument shows excellent accuracy for the different CRMs. For the elements As, Cd, Hg, and Pb, the recovery calculation is between 90% and 120% for the three sample preparations, thus ensuring excellent accuracy of the results on these highly toxic elements for children.

It should be noted that the recovery calculations are also extremely accurate for chromium and selenium, which are also elements that can cause problems with children's health. At the same time, accurate recoveries within the limit of ±20% were also obtained in CRM LGC7103, which shows that the workflow not only guarantees the results on toxic elements, but also meets the same requirements for essential and major elements necessary to be present at high concentrations.

Unknown sample analysis

The results obtained for the six unknown baby food products are summarized in Table 7 again with the results for highly toxic contaminants arsenic, cadmium, mercury, and lead highlighted.

Table 6. Certified values, concentration measured, and percentage recoveries of CRM TF002RM (* indicative value)

		LGC7103 - Sweet digestive biscuit			TF002RM - Skimmed milk powder			T07413QC - Infant cereal (rice based)		
Element	Unit	Certified value	Determined value	Recovery (%)	Certified value	Determined value	Recovery (%)	Certified value	Determined value	Recovery (%)
Na	$mg \cdot kg^{-1}$	5010 ± 400	5370 ± 207	107	-	-	-	-	-	-
Mg	$mg \cdot kg^{-1}$	254 ± 59	260 ± 7.1	103	-	-	-	-	-	-
P	$mg \cdot kg^{-1}$	900 ± 140	1001 ± 11	111	-	-	-	-	-	-
K	$mg \cdot kg^{-1}$	1580 ± 170	1729 ± 51	109	-	-	-	-	-	-
Ca	$mg \cdot kg^{-1}$	480*	537	112	-	-	-	-	-	-
Cr	$\mu g \cdot kg^{-1}$	-	-	-	-	-	-	127 ± 27	131 ± 6	103
Mn	$mg \cdot kg^{-1}$	5.49 ± 0.60	6.35 ± 0.07	115	-	-	-	-	-	-
Fe	$mg \cdot kg^{-1}$	18.0*	14.9	83	-	-	-	-	-	-
Cu	$mg \cdot kg^{-1}$	1.5*	1.4	93	-	-	-	-	-	-
Zn	$mg \cdot kg^{-1}$	6.41 ± 0.99	6.94 ± 0.12	108	-	-	-	-	-	-
As	$\mu g \cdot kg^{-1}$	-	-	-	72.9 ± 3.8	73.1 ± 6.3	100	113 ± 25	121 ± 12	107
Se	$\mu g \cdot kg^{-1}$	-	-	-	-	-	-	78.3 ± 17.2	81.2 ± 1.2	103
Cd	$\mu g \cdot kg^{-1}$	-	-	-	21.2 ± 0.7	23.3 ± 0.8	109	32.8 ± 7.2	36.4 ± 3.2	110
Hg	$\mu g \cdot kg^{-1}$	-	-	-	39.6 ± 2.3	41.1 ± 2.6	104	29.6 ± 6.5	28.3 ± 1.9	95
Pb	$\mu g \cdot kg^{-1}$				52.9 ± 3.4	57.8 ± 3.8	109	44.9 ± 9.9	48.9 ± 3.9	108

Table 7. Concentration of 28 elements in six baby food products. Highlighted are the concentrations obtained for As, Cd, Hg, and Pb.

	Yogurt ($\mu g \cdot kg^{-1}$)	Apple nectarine banana compote ($\mu g \cdot kg^{-1}$)	Carrot puree ($\mu g \cdot kg^{-1}$)	Salmon with vegetables ($\mu g \cdot kg^{-1}$)	Lamb puree ($\mu g \cdot kg^{-1}$)	Biscuit ($\mu g \cdot kg^{-1}$)
Al	1029.19	1864.70	319.32	2528.42	1521.18	1649.14
Ti	489.37	108.02	130.08	191.00	112.16	282.27
V	4.07	3.54	3.86	6.63	5.42	9.61
Cr	49.03	13.85	278.68	58.30	99.43	24.41
Mn	68.79	591.98	640.16	1802.16	1094.23	2763.63
Fe	1629.50	1423.99	2629.07	7551.37	5586.98	7833.95
Co	1.84	2.60	4.95	4.41	4.26	3.76
Ni	0.68	85.02	195.33	45.53	78.05	10.63
Cu	60.68	662.74	536.14	780.91	849.48	758.51
Zn	4907.49	879.45	3334.72	6663.95	5722.24	10968.06
As	0.45	2.13	2.12	23.21	1.98	4.61
Se	7.22	2.88	1.73	17.92	6.97	74.19
Rb	459.55	1503.24	753.22	521.30	1199.07	826.55
Sr	330.44	230.64	635.17	682.64	596.09	497.70
Mo	54.06	10.80	50.09	110.66	52.49	201.67
Ag	8.66	6.24	5.21	5.50	5.96	6.18
Cd	0.06	0.47	12.80	4.47	9.21	7.06
Sn	377.22	62.97	216.78	54.89	54.60	13.34
Sb	1.24	1.25	2.02	0.57	0.60	1.09
Te	0.00	0.48	1.38	0.00	1.91	0.84
Ba	90.60	201.09	1426.58	141.14	186.50	461.18
La	0.31	2.56	0.67	2.16	0.84	0.83
Ce	0.53	1.80	0.83	3.72	1.34	1.67
Hg	1.24	0.41	0.57	2.65	0.54	0.87
Tl	0.59	7.74	7.72	8.13	8.89	12.95
Pb	1.46	2.65	13.57	2.73	3.49	4.67
Bi	1.09	1.60	0.46	0.02	0.10	0.46
U	0.09	0.36	0.11	0.31	0.37	0.14

The results obtained were compared to the maximum levels of arsenic, lead, cadmium, and mercury allowed in baby food recommended by The Baby Food Safety Act of 2021 (Figure 3).

More than 23 $\mu g \cdot kg^{-1}$ of total arsenic was detected in the salmon and vegetables puree. While the maximum limit of 10 $\mu g \cdot kg^{-1}$ has been established for the highly toxic inorganic forms of As, it is not unusual to also find this element incorporated in organic molecules, especially in fish and seafood. These species are of far lower toxicity, so the overall amount found for As may not be of immediate concern. To verify the chemical form of As, it would be required to conduct speciation analysis using ion chromatography (IC) hyphenated to the iCAP MTX ICP-MS. The same sample was also found to slightly exceed the permitted amount of Hg. Again here, this is most likely related to salmon, as it is well-known that mercury can accumulate in the muscle tissues of fish following its absorption from surrounding waters or from the consumption of preys that contain mercury. Thus, it is not surprising to detect more than 2 $\mu g \cdot kg^{-1}$ in fish-based baby foods.

Three samples contained a cadmium concentration above 5 $\mu g \cdot kg^{-1}$. Cadmium is a metallic element that is very frequently found in the environment in its natural state, but due to industrial and agricultural activities it can also contaminate plants. It thus enters the food chain and can be found in baby food products.

Similarly, it is not surprising to find a lead concentration greater than 5 µg·kg-1 in the carrot puree since carrots are a vegetable that grows in the ground and, depending on the region, the soil can be loaded with lead.

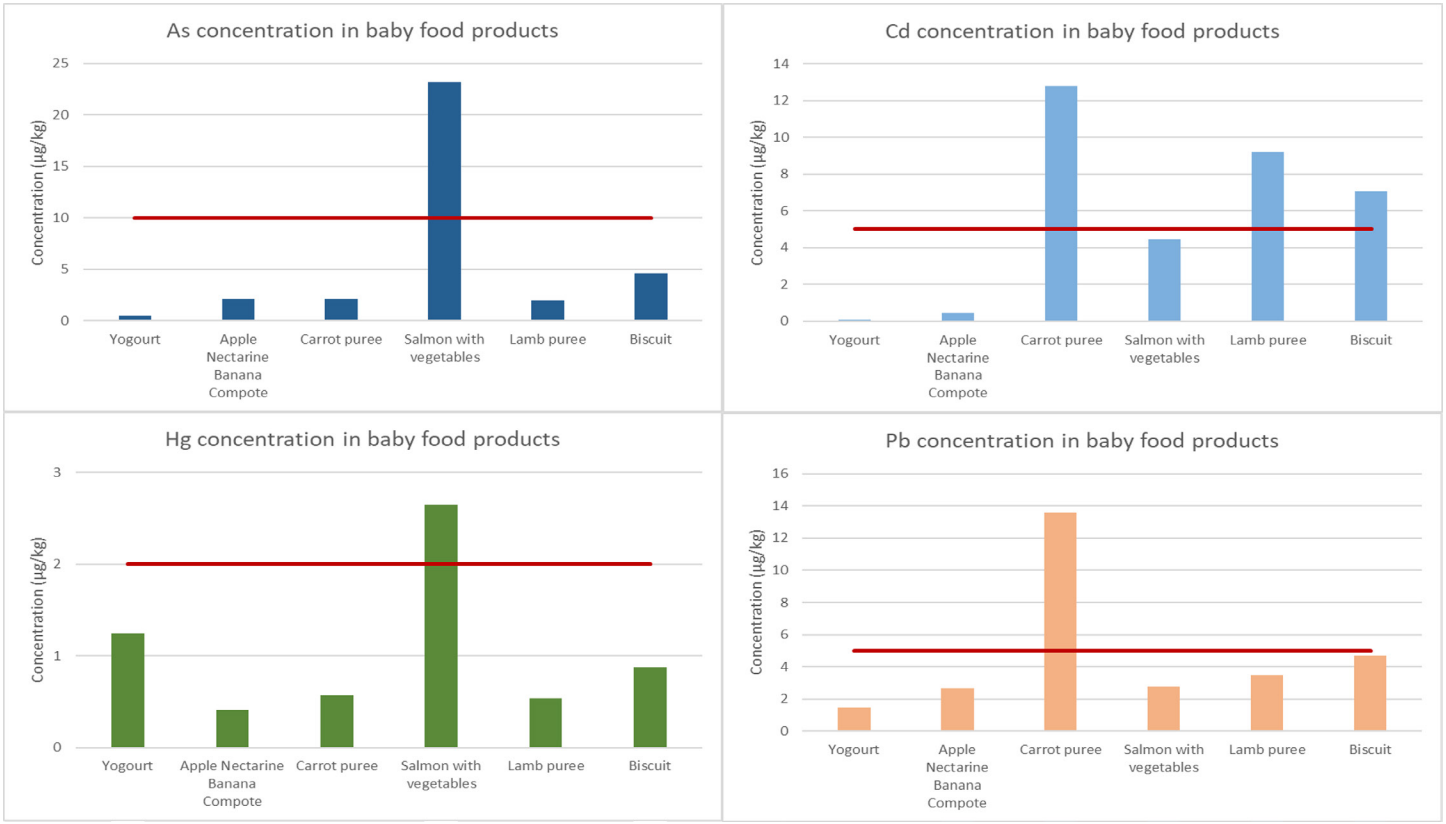

Figure 3. Concentration of As, Cd, Hg, and Pb compared with recommended maximum limits

Conclusion

This workflow, combining sample preparation using microwave assisted digestion and analysis using the iCAP MTX ICP-MS, allows accurate measurement of the concentration of toxic elements, dangerous for children, as well as the essential elements, necessary for the good growth and health of children, in various baby food products.

The automatic and constant 5-times dilution allows robust analysis of different types of baby food products, whether dry, liquid, or fatty, without any compromise on sensitivity to reach the regulated limits. Thanks to the iCAP MTX ICP-MS stability and reliability, a large variety of different food samples can be analyzed in the same run, ensuring productivity in an applied testing laboratory.

All types of interferences (polyatomic as well as isobaric interference, including doubly charged ions) are effectively suppressed with either helium collision gas or by using oxygen as a reactive gas in triple quadrupole mode, thus limiting unwanted interruptions of the analysis due to QC failures.

Qtegra ISDS Software with the Reaction Finder functionality, allows simplified handling for the operator by automatically defining the isotopes and the acquisition modes to obtain reliable results. The maintenance assistant also ensures alerts when maintenance actions need to be carried out by the operator in order to guarantee an instrument with the best performance throughout the life of the instrument.

References

1. Nóbrega, J.A.; Carnaroglio, D.; et al. Best practices in sample preparation of baby food for trace metal determination, Milestone Srl., 2022.
2. Sengupta, S.; Surekar, B.; Kutscher, D. Thermo Fisher Scientific, Application Note 000209 - Using triple quadrupole ICP-MS to improve the speed, sensitivity, and accuracy of the analysis of toxic and nutritional elements in baby foods, 2021. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-000209-icp-ms-toxic-and-nutritional-elements-baby-food-an000209-na-en.pdf
3. Raab, A.; McSheehy Ducos, S. Thermo Fisher Scientific, Application Note 43255 - Determination of inorganic arsenic in rice using IC-ICP-MS, 2018. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-43255-IC-ICP-MS-Inorganic-Arsenic-Rice-AN43255-EN.pdf

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