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Analysis of infant formulae and milk powders using the Thermo Scientific iCAP 7400 ICP-OES Duo

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Goal

Demonstrate the suitability of ICP-OES for determination of nutritional and certain toxic elements in infant formulae.

Introduction

For the first six months of life, breast milk is recommended as the sole source of infant nutrition. However, less than 35% of the world's infants are exclusively breast fed at this age. Infant formula is the most common substitute in the US and in Europe for infants younger than four months. Substitutes to breast milk must provide sufficient supplies of energy and nutrients to support the rapid growth rate during the infant's first six months of life. Inadequate or incorrect nutrient and energy intakes can directly affect infant growth and can have long-term consequences on organ development and function, which may result in adverse health effects later in life.

Commission directive 2006/141/EC lays down the essential composition of infant and follow-on formulae, including the minimum and maximum concentration of 13 mineral substances, of which 10 are potentially measurable by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Table 1).

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Table 1. Composition of mineral substances in infant formulae according to CD 2006/141/EC.

Element	Unit	Infant formulae per 100 kcal		Follow-on infant formulae per 100 kcal	
		Minimum	Maximum	Minimum	Maximum
Na	mg	20	60	20	60
K	mg	60	160	60	160
Ca	mg	50	140	50	140
P	mg	25	90	25	90
Mg	mg	5	15	5	15
Fe	mg	0.3	1.3	0.6	2
Zn	mg	0.5	1.5	0.5	1.5
Cu	µg	35	100	35	100
Se	µg	1	9	1	9
Mn	µg	1	100	1	100

Other regions and organizations that regulate the allowed mineral substance content in similar ways are e.g.

- Codex Alimentarius - Codex Stan 72-1981 (FAO/WHO)
- US FDA CFR 21 Part 107
- Indian Standard IS 14433:2006
- Chinese Standard GB 10765-2010

Additionally in some standards it is tested for toxic elements like lead, cadmium, tin or arsenic.

Table 2. Maximum allowed concentration of toxic elements in infant formula (mg·kg^{-1}).

Element	EU 1881-2006 (wet weight)	IS 14433-2006 (dry weight)	GB 2762-2012 (dry weight)
Pb	0.02	0.2	0.15
As	-	0.05	-
Cd	-	0.1	-
Sn	-	5	-

This application note will detail which elemental assessment is possible with the technique of ICP-OES.

Instrumentation

For all analyses, a Thermo Scientific™ iCAP™ 7400 ICP-OES Duo was used together with an aqueous sample introduction kit. A Teledyne CETAC ASX-560 Autosampler was used to automatically transfer the sample to the ICP-OES.

The iCAP 7400 ICP-OES Duo is perfectly suited for this type of application due to its high detection power for trace elements and its wide dynamic range for analysis of nutrients present at high concentrations. This allows to run all samples once and without further dilution. The Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software was used to perform the analysis and evaluate the data quantitatively. Qtegra ISDS Software contains dedicated feature sets that simplify method development and provide easy options for post-analysis data manipulation.

Standard and sample preparation

Samples

Three different infant and follow-on formulae were purchased in a local drug store. For comparison and validation of the procedure, a certified reference material (CRM) was additionally included in this study:

- Infant Formula (for newborns)
- Follow-On Milk (after six months)
- Milk for Children (after one year)
- CRM BCR-063R Skim Milk Powder

For each sample, duplicates were prepared to show reproducibility of the digestion. For the digestion, the powder was weighed (~0.5 g) into a PTFE high pressure vessel, and 7 mL of concentrated nitric acid (TraceMetal™ Grade, Fisher Chemical, Loughborough, UK) were added. Any material adhered to the walls of the vessel was washed down carefully with the acid. To increase the oxidation potential for decomposing the organic matrix, 1 mL of concentrated hydrogen peroxide (Primar™ Grade, Fisher Chemical, Loughborough, UK) was added as well. The digestion was conducted in a Milestone Ethos EZ microwave, equipped with an SK-10 segmented rotor and a temperature sensor, according to the protocol in Figure 1. After digestion and cooling down to room temperature, the digest was transferred to a 50 mL volumetric flask. The digestion vessels were rinsed with 18 MΩ ultra-pure water and the resulting liquid was transferred to the flask as well. Finally, the flask was filled up with ultra-pure water to the mark. Additionally, a duplicate of a digestion blank was run to collect evidence for contaminations occurring during the digestion procedure. The digestion blank contained the same amount of nitric acid and hydrogen peroxide as used for all samples and was treated the same way.

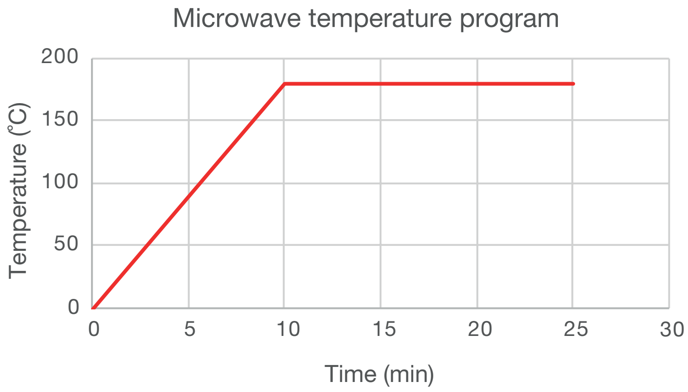

Figure 1. Temperature program of the digestion.

Standards

A standard stock solution was prepared from single element solutions (1000 mg·kg^{-1} and 10000 mg·kg^{-1}, SPEX CertiPrep Group, Metuchen, US) and then diluted to the concentrations displayed in Table 3. The individual standard solutions were made up with ultra-pure water and nitric acid to achieve a final acid concentration of 9.6% HNO_3 to match the acid content of the digested samples.

Table 3. Concentration of elements in the standard solutions used for calibration (mg·kg^{-1}).

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Pb	0.01	0.02	0.03	0.04	0.05	-
Na	10	20	30	40	50	-
K	40	80	120	160	200	-
Ca	30	60	90	120	150	-
P	20	40	60	80	100	120
Mg	3	6	9	12	15	-
Fe	0.02	0.04	0.06	0.08	0.1	1
Zn	0.2	0.4	0.6	0.8	1	-
Cu	0.02	0.04	0.06	0.08	0.1	-
Se	0.006	0.012	0.018	0.024	0.03	-
Mn	0.01	0.02	0.03	0.04	0.05	-
Mo	0.006	0.012	0.018	0.024	0.03	-
Cr	0.02	0.04	0.06	0.08	0.1	-
Sn	0.02	0.04	0.06	0.08	0.1	-

Method development

The typical operation parameters for the iCAP 7400 ICP-OES Duo can be found in Table 4. After ignition of the plasma the instrument was allowed to warm up for 15 minutes. A spectrometer optimization was performed directly before the analysis.

Table 4. Instrument parameters.

Parameter	Setting	
Pump Tubing (Standard Pump)	Sample Tygon® orange/white Drain Tygon® white/white	
Analysis Pump Speed	50 rpm	
Spray Chamber	Glass Cyclonic	
Nebulizer	Glass Concentric	
Nebulizer Gas Flow	0.5 L min^{-1}	
Coolant Gas Flow	12 L min^{-1}	
Auxiliary Gas Flow	0.5 L min^{-1}	
Center Tube	2 mm	
RF Power	1150 W	
Plasma View	**Axial**	**Radial**
Exposure Time	UV 15 s, Vis 5 s	Vis 5 s

A method was created in Qtegra ISDS Software using the wavelengths shown in Table 5 for the elements under investigation. The selected wavelengths were free from direct spectral interferences and provided the sensitivity to quantify the elements of interest in the expected concentration range.

Following method development, the instrument was calibrated and the samples were analyzed. A detection limit study was carried out by analyzing the calibration blank three times with ten replicates. The resulting instrument detection limit (IDL) was calculated based on the 3σ criterion. Additionally, the method detection limit (MDL) was determined by applying the sample dilution factor of 100. This gives an indication of the detection limit in the solid material.

Table 5. Analyte wavelength and view as well as IDL and MDL, and the correlation coefficient of the calibration curve (R^2). Please note the different units for IDL and MDL values.

Element and wavelength (nm)	View	R^2	IDL (µg·kg^{-1})	MDL (mg·kg^{-1})
Pb 220.353	Axial	0.9991	2.2	0.22
Na 589.592	Radial	1.0000	28	2.8
K 766.490	Radial	1.0000	75	7.5
Ca 317.933	Radial	0.9999	7.7	0.77
P 213.618	Axial	0.9998	3.4	0.34
Mg 279.553	Radial	0.9998	0.13	0.013
Fe 259.837	Axial	1.0000	2.4	0.24
Zn 202.548	Axial	0.9998	0.12	0.012
Cu 224.700	Axial	0.9996	0.7	0.07
Se 196.090	Axial	0.9974	5.1	0.51
Mn 257.610	Axial	0.9997	0.10	0.01
Mo 202.030	Axial	0.9998	0.64	0.064
Cr 205.560	Axial	0.9998	0.47	0.047
Sn 189.989	Axial	0.9999	0.56	0.056

Results and discussion

For most elements, the duplicate analysis of the reference material shows excellent recovery between 95 and 101%, with low RSDs between analysis duplicates (Table 6). Molybdenum shows a slightly lower recovery of 84%, however Mo is not certified in BCR-063R, only an information value is provided in the certificate of analysis. Therefore, no accurate conclusions can be drawn from this result.

Table 6. Recovery of the certified reference material and precision of the duplicate analysis.

Element	Certified reference concentration (mg·kg^{-1})	BCR-063R replicate 1		BCR-063R replicate 2		RSD (%)
		Concentration (mg·kg^{-1})	Recovery (%)	Concentration (mg·kg^{-1})	Recovery (%)	
Pb	0.0185 ± 0.0027	< MDL	-	< MDL	-	-
Na	4370 ± 31	4225	96.7	4178	95.6	0.8
K	17680 ± 190	16995	96.1	16804	95.0	0.8
Ca	13490 ± 100	13360	99.0	13084	97.0	1.5
P	11100 ± 130	11256	101.4	11135	100.3	0.8
Mg	1263 ± 24	1219	96.5	1213	96.0	0.3
Fe	2.32 ± 0.23	3.4	146.6	2.9	123.0	11.2
Zn	49 ± 0.6	48.7	99.4	48.2	98.5	0.7
Cu	0.602 ± 0.019	0.58	96.6	0.60	100.4	2.4
Se	0.129*	< MDL	-	< MDL	-	-
Mn	-	0.26	-	0.26	-	0.0
Mo	0.33*	0.28	84.0	0.27	82.4	2.6
Cr	-	< MDL	-	< MDL	-	-
Sn	-	< MDL	-	< MDL	-	-

*information values
< MDL: below method detection limit

Iron shows high recoveries at 146 and 123%, respectively. As the concentrations were well above the method detection limit, it is unlikely that elevated recoveries are influenced through the overall measurement uncertainty. As a potential contamination source, all acids and reactants used for digestion were analyzed separately, but did not show an elevated value (Table 7), hence cannot explain these increased levels. To exclude errors in the preparation of the calibration solutions or interferences as the cause for this out-of-range recovery, the method of standard addition was applied. The samples were spiked with single element iron solutions at multiples of the concentration of the expected value. This resulted in the same concentration as compared to external calibration, therefore measurement and calibration errors can be excluded. The final cause of the elevated recovery remains unknown, but it is likely that a combination of contamination of the material as well as inhomogeneity are the reasons, as the duplicate analysis showed a strong deviation between individually prepared samples. However, since iron is accurately measured as indicated by the application of the method of standard addition, iron concentrations at the expected level can in general be measured accurately with this method.

The relative standard deviation (RSD) between replicates for all other analytes is in the range of 0.5 to 2.3%, likely resulting from slight inhomogeneity of the subsample, however indicating good reproducibility of the microwave digestion method.

In Tables 7, 8 and 9, the results of the analyzed infant and follow-on formulae used in this study can be found. Potential contaminations from the sample preparation procedure were found to be negligible but are subtracted from the individual results. The tables contain the average concentration, RSD between analysis duplicates and a reference value where available (for example, according to the description on the package). For better comparability to regulated values in Table 1, the average concentration was calculated in mg per 100 kcal, according to the amount of powder used to make a solution containing 100 kcal.

Table 7. Results of the analyzed digestion blank and *Infant Formula*.

Element	Blank		Infant Formula			
	Average concentration (mg·kg^{-1})	RSD (%)	Reference value (mg·kg^{-1})	Average concentration (mg·kg^{-1})	Recovery (%)	Average concentration (mg per 100 kcal)
Pb	< MDL	-	-	< MDL	-	< MDL
Na	8.7	44	1250	1363	109.0	28
K	13	20	5294	5108	96.5	105
Ca	4.1	52	4044	3651	90.3	75
P	< MDL	-	2279	2477	108.7	51
Mg	0.1	41	375	353	94.1	7.2
Fe	< MDL	-	39.0	40.3	103.3	0.83
Zn	0.06	8	37.5	38.7	103.2	0.80
Cu	< MDL	-	2.94	3.12	106.1	0.064
Se	< MDL	-	0.125	< MDL	-	< MDL
Mn	< MDL	-	0.588	0.568	96.6	0.012
Mo	< MDL	-	-	< MDL	-	< MDL
Cr	< MDL	-	-	< MDL	-	< MDL
Sn	< MDL	-	-	< MDL	-	< MDL

Table 8. Results of *Follow-On Milk*.

Element	Follow-On Milk			
	Reference value (mg·kg^{-1})	Average concentration (mg·kg^{-1})	Recovery (%)	Average concentration (mg per 100 kcal)
Pb	-	< MDL	-	< MDL
Na	1418	1796	126.6	36
K	5745	6285	109.4	127
Ca	5035	5244	104.2	106
P	3050	3359	110.1	68
Mg	440	483	109.8	9.7
Fe	70.9	69.6	98.2	1.4
Zn	35.5	38.1	107.3	0.77
Cu	2.8	2.5	89.3	0.049
Se	0.11	< MDL	-	< MDL
Mn	0.54	0.54	100.0	0.011
Mo	-	0.223	-	0.004
Cr	-	< MDL	-	< MDL
Sn	-	< MDL	-	< MDL

Table 9. Results of *Milk for Children*.

Element	Milk for Children			
	Reference value (mg·kg^{-1})	Average concentration (mg·kg^{-1})	Recovery (%)	Average concentration (mg per 100 kcal)
Pb	-	< MDL	-	< MDL
Na	-	1855	-	41
K	-	7954	-	175
Ca	10818	10383	96.0	228
P	-	5854	-	129
Mg	-	551	-	12.1
Fe	-	1.5	-	0.033
Zn	-	20.1	-	0.44
Cu	-	0.21	-	0.005
Se	-	< MDL	-	< MDL
Mn	-	0.902	-	0.020
Mo	-	0.179	-	0.004
Cr	-	< MDL	-	< MDL
Sn	-	< MDL	-	< MDL

Most results fall within ±10% of the corresponding reference value. Only *Follow-On Milk* (Table 8) shows a slightly elevated level of sodium (126%), however all concentrations obtained for *Infant Formula* and *Follow-On Milk* are in the acceptable concentration range according to the regulatory guidelines shown in Table 1. An exception is selenium since the achieved detection limits are not sufficient to determine the concentration in the expected concentration range, therefore another detection technique needs to be applied for this element. The results for *Milk for Children* are exceeding the maximum allowed concentrations for some elements (K, Ca, P) and are lower than the minimum value for others (Fe, Zn, Cu). However, this product is intended for use with children older than one year and therefore does not fall under the regulation of mineral substances in infant formula in Commission Directive 2006/141/EC (Article 2).

Conclusion

The analysis shows that the Thermo Scientific iCAP 7000 Plus Series ICP-OES delivers excellent robustness and accuracy for analyses of nutritional components in infant formulae in conformity with the present Commission Directive 2006/141/EC and other global regulations. Moreover, the very good RSDs of the duplicate analysis indicate that no target elements are lost demonstrating microwave digestion is an excellent choice for sample preparation.

When wanting to quantitatively analyze toxic elements like cadmium or tin, ICP-OES may be a suitable technique (Table 10). However, for trace elements like selenium, lead or arsenic, the detection limits achieved with ICP-OES do not suffice the requirements of individual regulations (Tables 1 and 2) due to the high dilution factor applied in sample preparation. For these elements, other techniques like ICP-MS or GF-AAS as delivered through the Thermo Scientific™ iCAP™ RQ ICP-MS or the Thermo Scientific™ iCE™ 3500 GF-AAS need to be applied.

Table 10. Comparison of maximum values for toxic elements in infant formulae (Table 2) and detection limits achievable with the iCAP 7000 Plus Series ICP-OES.

Element	Maximum value ($mg\cdot kg^{-1}$ dry weight)	Detection limit in sample ($mg\cdot kg^{-1}$)
Pb	0.15	0.22
As	0.05	0.36*
Cd	0.1	0.01*
Sn	5	0.056

*Detection limits established in a comparable matrix, see application note AN43263[1]

References

1. S. Asendorf. Analysis of trace elements in Traditional Chinese Medicine (TCM). Thermo Fisher Scientific Application Note 43263. Bremen, Germany, 2017.

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