Application Note
Food

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Determination of Nutrients and Micronutrients in Milk Powder and Infant Formula by ICP-OES

Quality assured results per ISO 15151 method using the Agilent 5800 ICP-OES with smart tools

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Introduction

Milk is an important source of the fats, vitamins, minerals, nutrients, and micronutrients needed for a balanced diet. It is also vital for an infant's survival, growth, and development. Manufacturers of milk powder and infant formula products aim to replicate the nutritional components of milk in an easy-to-use form. However, all infant formula products must be produced in accordance with strict nutrient requirements that are specified by regional, national, or international regulatory bodies. Manufacturers must also ensure that no contamination occurs during the processing of their products. Traditionally, these types of samples are typically analyzed using Flame Atomic Absorption Spectroscopy (FAAS). However, the need for lower detection limits and higher sample throughput has pushed users and regulatory bodies towards faster, multi-element analytical techniques, such as ICP-OES.

Testing methods that are suitable for the determination of multiple-elements in manufactured milk products can be adapted from standard methods such as ISO 15151:2018 (*1*). ISO 15151 defines limits for the levels of major elements and essential elements in milk, milk products, infant formula, and adult nutritionals by ICP-OES.

Typically, food quality assurance/quality control (QA/QC) labs process large numbers of samples. Ensuring an accurate result with the first measurement is critical, as any sample remeasurements require analyst time and extra resources, adding to the costs of the analysis. To address these issues, the Agilent 5800 Vertical Dual View (VDV) ICP-OES uses a series of smart tools to assist with method development, validation, and data accuracy. For example:

- The IntelliQuant function within the Agilent ICP Expert instrument control software provides another data set for every sample within seconds (*2, 3*). Comparing the two data sets (quantitative and semiquantitative) is a quick and easy way to QA the results. If needed, IntelliQuant can also be used to troubleshoot the cause of any outlying data.
- The early maintenance feedback (EMF) function uses a series of sensors and counters to ensure that maintenance is completed only when required. Keeping the instrument in optimal condition with ongoing monitoring improves instrument performance and stability. It also minimizes the need to rerun QC samples due to an issue arising with the instrument.
- The IntelliQuant Screening software function allows users to start collecting data within seconds, without having to choose which elements or wavelengths to analyze (*4*). IntelliQuant Screening helps with method development. By automatically identifying the presence of any spectral interferences, IntelliQuant Screening recommends the best analyte wavelengths to use for each element.

The Agilent ICP Expert instrument control software for the 5800 ICP-OES provides an easy-to-follow workflow, which helps analysts who are familiar with other techniques, such as FAAS, to switch to Agilent ICP-OES. The IntelliQuant function is helpful for all analysts, but especially less experienced users, as it provides extra sample insight for all samples. IntelliQuant and IntelliQuant Screening show which elements are present in the samples in real time, including any contaminant elements introduced during the manufacturing process. For example, for infant formula products, if silicon leaks from pipes used in the freeze-drying process, it can spoil whole product-batches (*5*).

In this study, an Agilent 5800 VDV ICP-OES with an SPS 4 autosampler was used to determine Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn in milk powder and infant formula samples according to the ISO 15151 method.

Experimental

Instrumentation

The Agilent 5800 VDV ICP-OES was fitted with a SeaSpray nebulizer, double-pass glass cyclonic spray chamber, and semi-demountable dual view (DV) 1.8 mm i.d injector torch. The Agilent SPS 4 autosampler was used for the fast and automated delivery of the samples to the ICP-OES. The internal standard was added on-line using a Y-piece connection to the nebulizer. Agilent ICP Expert smart software functions including IntelliQuant and EMF were used to simplify the analysis, avoid sample remeasurements, and ensure optimum analytical performance. Instrument operating parameters are shown in Table 1.

Table 1. 5800 VDV ICP-OES instrument and method parameters.

Parameter	Axial	Radial
Read time (s)	10	5
Replicates	3	
Sample Uptake Delay (s)	16	
Stabilization Time (s)	15	0
Rinse Time (s)	30	
Pump Speed (rpm)	12	
Fast Pump During Uptake and Rinse	Enabled	
RF Power (kW)	1.2	
Auxiliary Flow (L/min)	1.00	
Plasma Flow (L/min)	12.0	
Nebulizer Flow (L/min)	0.7	
Viewing Height (mm)	16	
Sample Pump Tubing	White-white	
Internal Standard Tubing	Orange-green	
Internal Standard	5 ppm yttrium	
Waste Pump Tubing	Blue-blue	
Background Correction	Fitted	

Background correction

The ICP Expert software includes easy-to-use background correction techniques including Fitted Background Correction (FBC), which is suited to background correction of both simple and complex background peaks. Since interferences are likely to arise from background structures during the analysis of food samples, FBC was used in this study. The combination of the FBC algorithm and Vista Chip III detector accurately corrects background structures without user intervention or method development. An example of automatic background fitting using FBC is shown in Figure 1. FBC provides accurate correction of the background structure arising from an OH emission line, allowing the low-level detection of Cu 327.395 nm.

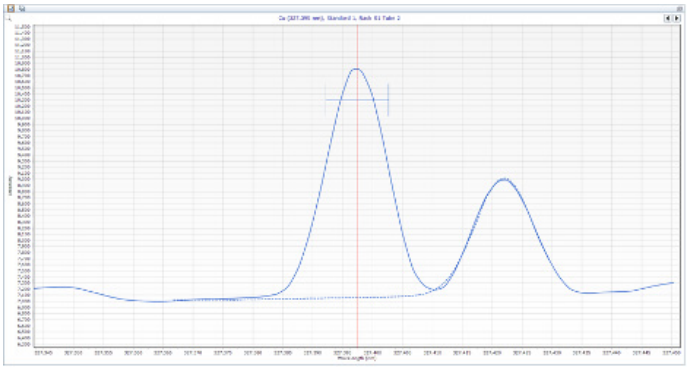

Figure 1. Automatic background correction using FBC for Cu 327.395.

Automatic instrument performance checking

Insufficient maintenance of an ICP-OES can lead to costly unplanned downtime or analysis failures that lead to time-consuming sample remeasurement. Maintenance that is too frequent also wastes time and can increase expenditure on consumables. The 5800 instrument's EMF function comprises a series of sensors and maintenance counters that alert the analyst when maintenance is needed. User-configured or default counter settings can be used for specific sample types.

This EMF system reduces downtime and repair costs by scheduling routine maintenance of components based on actual usage, rather than at set time intervals. EMF alerts the analyst of a problem and then guides them through the process of fixing it (using online help routines), avoiding the inconvenience of downtime or a service call. Ongoing instrument monitoring also ensures that data precision and data accuracy are maintained.

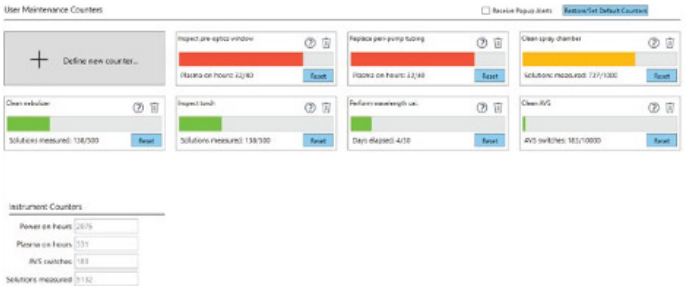

Figure 2. Early maintenance feedback counters.

Standard and sample preparation

Milk powder, powdered infant formula, liquid ready-to-use infant formula, and infant formula concentrate products were used in this study. A trace element in a milk powder certified reference material (CRM) MP-A (High Purity Standards) was used as part of the method evaluation. The samples and CRM were prepared for analysis using microwave digestion. First, 1 g of the samples were predigested for 15 minutes at room temperature in 10 mL HNO_3. They were then digested using a Mars 6 Microwave Digestion System (CEM Corporation, NC, USA) per the *infant formula* one-touch program outlined in Table 2. The resulting digested sample was then diluted to a final volume of 50 mL.

Table 2. Microwave digestion parameters.

Parameter	Value
Power (W)	1030–1800
Temperature (°C)	200
Ramp Time (min)	20:00–25:00
Hold Time (min)	15:00

Method development

The IntelliQuant Screening software function was used to select wavelengths and quickly determine the approximate concentrations of each element in a sample (*4*). Using the smart IntelliQuant Screening feature it was determined that strontium (Sr) was present in all the samples. ISO 15151 suggests Sr as a possible internal standard for atomic lines; however, due to its presence in the samples it was not used. The calibration standards were prepared from Agilent single element calibration standards using a matrix of 20% nitric acid (Emsure, Merck). 18.2 MΩ de-ionized (DI) water (Merck Millipore) was used to dilute all solutions. A QC solution was prepared at one half of the highest concentration standard used for each element.

ISO 15151 specifies the upper concentration limit for each element. It also states that the calibration correlation coefficient for each wavelength should be greater than 0.9995. The 5800 VDV ICP-OES exceeded the ISO method requirements, as shown by the highest calibration standard and the correlation coefficient of >0.99993 for each wavelength (Table 3).

Table 3. Plasma viewing mode, background correction method, internal standard, and calibration information.

Element, Wavelength (nm)	Background Correction	Calibration Concentration Range (ppm)	ISO 15151 Upper Limit (mg/L)	Correlation Coefficient	Viewing Mode	Internal Standard
Ca 317.933	Fitted	0–350	256	0.99998	Radial	Y (371.029)
Cu 327.395	Fitted	0–0.25	0.24	0.99995	Axial	Y (371.029)
Fe 238.204	Fitted	0–5	4	0.99999	Axial	Y (371.029)
K 766.491	Fitted	0–400	400	0.99993	Radial	None
Mg 280.270	Fitted	0–50	22	0.99994	Radial	Y (371.029)
Mn 259.372	Fitted	0–0.25	0.2	0.99999	Axial	Y (371.029)
Na 589.592	Fitted	0–250	170	0.99994	Radial	None
P 213.618	Fitted	0–250	160	0.99998	Radial	None
Zn 202.548	Fitted	0–5	3.6	0.99991	Axial	Y (371.029)

Table 4. LOQs for each element (in sample) compared to lower limits specified in ISO 15151 method, n=6.

Element and Wavelength (nm)	ISO 15151 Lower Limit (mg/kg)	LOQ (mg/kg)
Ca 317.933	200	0.73
Cu 327.395	0.3	0.05
Fe 238.204	5	0.04
K 766.491	100	13.02
Mg 280.270	30	0.03
Mn 259.372	0.1	0.01
Na 589.592	100	1.83
P 213.618	150	2.39
Zn 202.548	2	0.02

Table 5. Recoveries for certified and reference elements in CRM MP-A. n=9.

Element and Wavelength (nm)	Certified Value (mg/kg)	Measured Value (mg/kg)	Recovery (%)
Ca 317.933	11400	11237	99
Cu 327.395	0.52	0.51	98
Fe 238.204	2.1	2.1	102
K 766.491	17600	16886	96
Mg 280.270	1130	1089	96
Mn 259.372	0.17*	0.18	106
Na 589.592	4340	4236	98
P 213.618	9340	9740	104
Zn 202.548	42	40	96

** Indicates a reference value. NA = not applicable*

Results and discussion

Limits of quantification

The limits of quantification (LOQs) were based on 10 sigma of 10 repeated measurements of the method blank (20% HNO_3). The results are an average of six runs performed on two separate instruments. The LOQs for each element (reported as mg/kg) are given in Table 4. The 5800 LOQs are all significantly lower than the lower limits specified in the ISO 15151 method, confirming the suitability of the instrument for the application.

CRM analysis

Digests of three milk powder CRM samples were analyzed three times using the 5800 VDV ICP-OES. The measured results (reported in mg/kg) are given in Table 5. The recoveries for all analytes with certified or reference values were within ±10% of the expected value.

IntelliQuant results

During the quantitative analysis of the CRM digests IntelliQuant was enabled. Table 6 shows a comparison of the certified and semiquantitative measured concentrations for nine of the analytes in the milk powder CRM. Typically, semiquantitative measurements are within ±30% of the expected value. However, in this study, all IntelliQuant values were within ±14% of the expected concentration. The unique combination of the Vista Chip III detector and comprehensive wavelength database allows IntelliQuant to deliver real-time sample insights based on each individual spectrum. Ensuring the selection of the best interference-free wavelengths leads to better-quality data.

Table 6. IntelliQuant results for certified and reference elements in the milk powder CRM.

Element	IntelliQuant Measured Value (mg/kg)	Certified Value (mg/kg)	Recovery (%)
Ca	10044	11400	88
Cu	0.49	0.52	95
Fe	2.1	2.1	98
K	15899	17600	90
Mg	1083	1130	96
Mn	0.19	0.17*	114
Na	3814	4340	88
P	9566	9340	102
Zn	44	42	105

** Indicates a reference value*

Long-term stability

To assess the stability of the 5800 VDV ICP-OES, 250 solutions, comprising digested infant formula, ready-to-eat infant formula, concentrate infant formula, and milk powder samples, were measured over 11-hours without recalibrating. During the analytical run, a QC solution was measured straight after the calibration standards then after every 10 samples. The average sample-to-sample analysis time was 150 s. The QC stability plot in Figure 3 shows the recovery of all elements over 11 hours to be within ±10%. The relative standard deviation (%RSD) was less than 0.9% for all elements (Table 7). The results demonstrate the excellent robustness and precision of the 5800 VDV ICP-OES for the routine QC analysis of infant formula and milk powder samples. The instrument was stable for more than 11 hours and since there were no QC failures, no samples were remeasured.

Table 7. Concentration and %RSD of the QC sample analyzed periodically throughout the long-term stability test.

Element and Wavelength (nm)	Concentration (ppm)	%RSD
Ca 317.933	175	0.5
Cu 327.395	0.125	0.8
Fe 238.204	2.5	0.7
K 766.491	200	0.9
Mg 280.270	25	0.8
Mn 259.372	0.125	0.8
Na 589.592	125	0.7
P 213.618	125	0.9
Zn 202.548	2.5	0.8

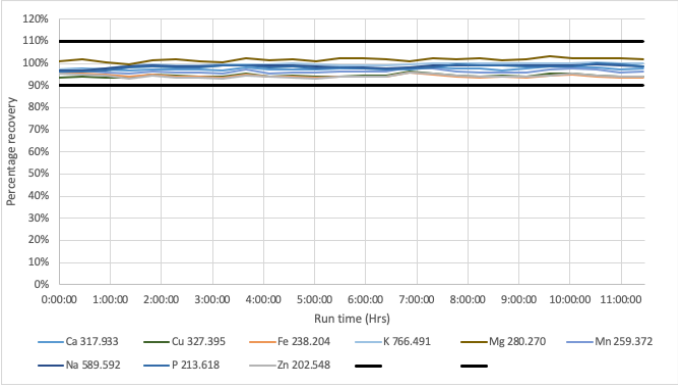

Figure 3. Long-term stability test showing recovery of a QC solution analyzed every 10 samples over 11 hours.

Conclusion

Accurate, routine measurement of elements in milk powder and infant formula and can be carried out using the Agilent 5800 VDV ICP-OES with an SPS 4 autosampler in accordance with the ISO 15151 method.

The smart tools included in the Agilent ICP Expert software add an extra layer of quality assurance to the analysis. The IntelliQuant and IntelliQuant Screening features assisted with method development and result validation, reducing the need to re-analyze any samples, and increasing confidence in the quantitative results. Other smart tools, including EMF, were used to maintain instrument performance and avoid downtime during the analysis of the food samples. FBC easily corrected the background structures that are typical of food-matrices.

The 5800 ICP-OES LOQs were below the lower limit requirements set out in the ISO 15151 method. Also, the instrument's calibration range and linearity showed that the upper limits specified in the method could be easily reached.

The high performance of the 5800 VDV ICP-OES was demonstrated by the recovery data for nine elements in a milk powder CRM. All recoveries were within ±10% of the certified or reference values. The 5800 also maintained excellent stability over the 11-hour QC recovery test, demonstrating the suitability of the method for the routine measurement of milk powder and infant formula.

References

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Note: The Agilent 5800 VDV ICP-OES requires ICP Expert Pro pack software to access IntelliQuant Screening. ICP Expert Pro pack software is standard with the Agilent 5900 SVDV system.

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