

Semi-quantitative analysis of nutrients in milk-based powders by EDXRF and UniQuant software

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Introduction

The food industry employs various analytical techniques to assess the nutritional value of milk-based powders, such as infant formula, which are enriched with added nutrients. These fortified milk powders contain proteins, fats, carbohydrates, vitamins, and minerals.

Energy-dispersive X-ray fluorescence (EDXRF) is utilized near the production line to monitor the elemental content of milk-based powders. Its quick and simple sample preparation, along with short measurement times, helps optimize production processes and reduce turnaround time. A previous application note ([XRF-AN41967](#)) already discussed the use of empirical calibrations in combination with multiple standards to setup a method which determines Na, Mg, P, Cl, K, Ca, Mn, Fe, Cu, and Zn.

When an extensive set of standards isn't available, a standardless fundamental parameters approach can still perform a reliable (semi-)quantitative analysis. In this AN we show how the Thermo Scientific™ UniQuant standardless fundamental parameters software package is used in combination with the Thermo Scientific™ ARL™ QUANT'X EDXRF Spectrometer to determine nutrients in milk-based powders.



ARL QUANT'X EDXRF Spectrometer.

UniQuant software

UniQuant software is designed for use with X-ray fluorescence (XRF) spectrometers. It provides a comprehensive solution for the quantitative analysis of a wide range of elements in various sample types without the need for specific calibration standards. UniQuant software utilizes fundamental parameters to analyze the XRF spectra, allowing for the accurate determination of elemental concentrations in complex and unknown samples. This makes it particularly useful for applications where sample composition varies widely or where reference materials are not available.

Instrumentation

The ARL QUANT'X EDXRF Spectrometer features a 50-Watt end-window X-ray tube with a Ag target. The sample is analyzed using direct excitation geometry and primary beam filters. There are 9 filters available to optimize element excitation. A large-area SDD with a graphene window, less than 1 μm thick, enables the detection of all elements in the periodic table from carbon ($Z = 6$) to americium ($Z = 95$). Previously, the default SDD window was an 8 μm thick Be window, which could not detect elements lighter than fluorine ($Z = 9$).

Sample preparation

6.0 ± 0.1 g of powder was pressed into a 32 mm diameter pellet using a die set made of hardened stainless steel. A manual hydraulic press (Specac, LTD, United Kingdom) was used to apply a pressure of 4 tons. Samples were not ground or dried prior to pelletizing. Pellets were stored in a desiccator.

The resulting pellets had a thickness of >5 mm. This can be considered infinitely thick with respect to the penetration depth of the characteristic X-rays of interest for each element. Therefore, pellets were analyzed on both sides and treated as real duplicates.

Condition	Voltage (kV)	Current (mA)	Filter	Atmosphere	Live Time (s)	Analytes
Group 9	4	2.00 (Auto)	No Filter	Vacuum	180	Na, Mg
Group 8	7	2.00 (Auto)	C Thin	Vacuum	180	P
Group 7	8	2.00 (Auto)	C Thick	Vacuum	60	Cl, K, Ca
Group 6	12	2.00 (Auto)	Al	Vacuum	60	Mn
Group 5	16	2.00 (Auto)	Ag Thin	Vacuum	60	Fe
Group 4	20	2.00 (Auto)	Ag Medium	Vacuum	180	Cu, Zn

Table 1. Excitation conditions used in this application note.

Measurement conditions

UniQuant software in combination with the ARL QUANT'X EDXRF Spectrometer uses 9 conditions in total to cover all detectable elements of the periodic table. Every condition is optimized to excite a range of elements. Milk-based powders do not contain more than 10 to 12 elements beside the organic matrix which consists mostly of lactose. Therefore, not all 9 conditions need to be run to cover all elements of interest. **Table 1** shows the experimental details of each excitation condition. Only 6 of the 9 conditions are necessary to cover all elements of interest. Measurements are run in vacuum to avoid absorption of low-energy characteristic X-rays emitted by lighter elements. Identical measurement times as in [XRF-AN41967](#) were used so that performance of each analytical approach can be compared directly.

The total measurement time consisted of 720 s “live time” with 180 s for three conditions that either focus on light elements or transition elements present at concentrations close to the detection limit (Mn and Cu). The measurement time can be adjusted according to customer expectations.

Calibration

The fundamental parameters model of UniQuant software is calibrated using a variety of pure elements, pure compounds, and certified reference materials. This general calibration can be used to determine the composition of an unknown sample containing elements at any concentration. This universal calibration provides semi-quantitative analysis results. The expected relative error is in the order of 15 % to 30 % and depends on the type of sample as well as the sample preparation.

To improve the accuracy, it is possible to adjust the calibration for a specific application. This is done by calibrating UniQuant software using standards which are similar to the unknown samples to be analyzed. For this application note we optimized UniQuant software's element sensitivities (so-called kappas) using a small set of 13 in-house standards previously analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) and potentiometry for chlorine especially (it was assumed that whole chlorine was chloride, disregarding all other molecules). **Table 2** shows the concentration range for each element; standards are measured in duplicate (both sides of the pellet).

These standards are also used to derive a spectral background shape which is used to correct characteristic element lines for the spectral background and obtain net peak intensities. Such a shape is used by UniQuant software to improve accuracy and precision for lower concentrations, especially concentrations close to the detection limits.

The matrix of these milk-based powders is assumed to be lactose. Correct characterization of the matrix is important for a correct calculation of the sample composition, especially when the matrix consists of elements which are difficult or impossible for EDXRF to detect, as is the case with an organic matrix consisting of C, H, N and O. UniQuant uses the formula of lactose to estimate and correct for the absorption by the matrix of the quantifiable elements. Incorrect characterization of the matrix might lead to biased concentrations and explains in part why an empirical calibration approach gives better results.

Element	Conc. range, mg/100g
Na	140 - 492
Mg	36 - 121
P	167 - 516
Cl	295 - 583
K	429 - 1125
Ca	255 - 757
Mn	0.3 - 1.3
Fe	5 - 10
Cu	0.3 - 0.8
Zn	3 - 10

Table 2. Elemental concentration ranges.



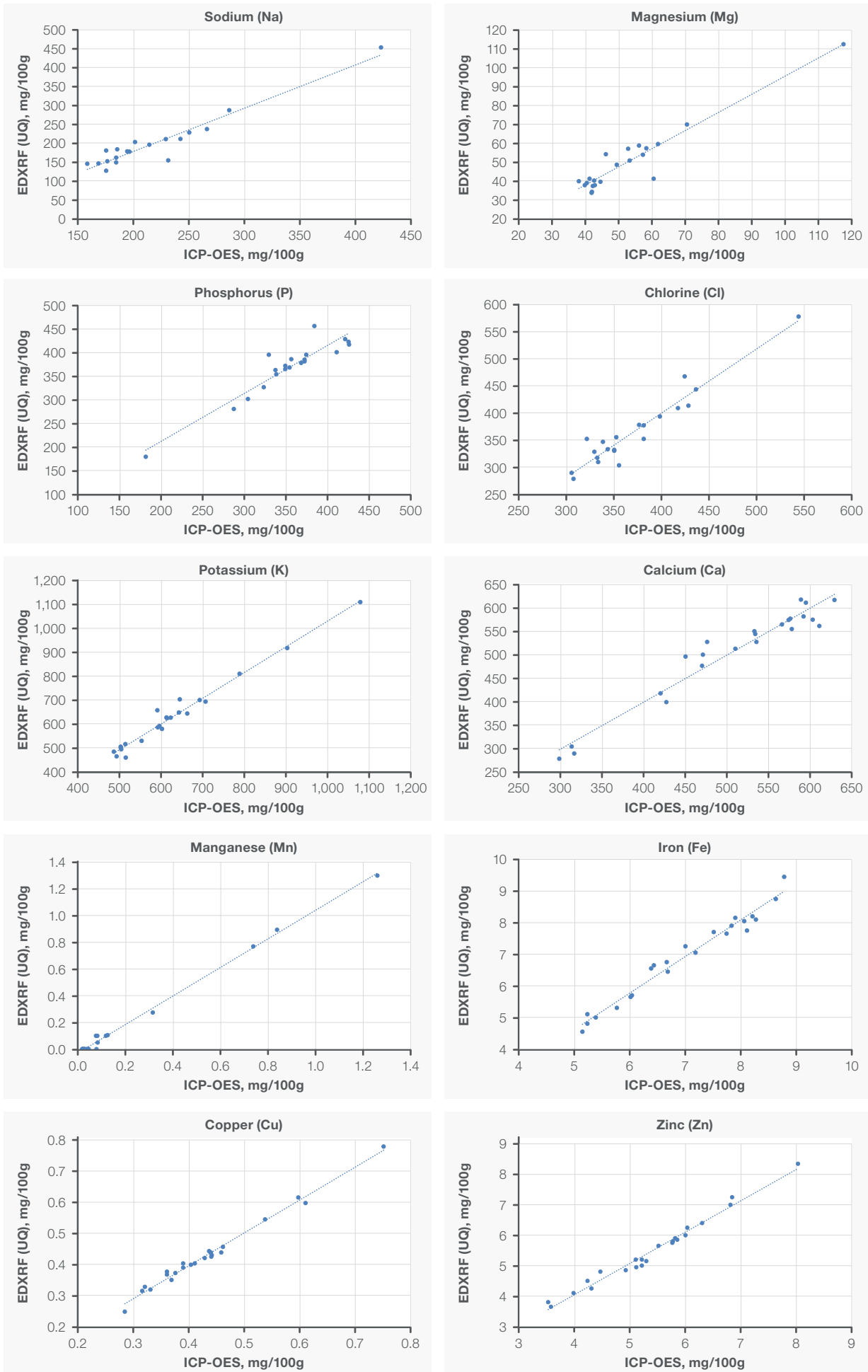


Figure 1. Milk-based powder standards analyzed with EDXRF & UniQuant software, as compared to ICP-OES and potentiometry results.

	Na	Mg	P	Cl	K	Ca	Mn	Fe	Cu	Zn
N	19	21	20	21	23	23	23	23	23	23
SEP	20	5	21	19	24	24	0.022	0.23	0.013	0.16
R²	0.920	0.907	0.869	0.917	0.975	0.942	0.996	0.970	0.987	0.979
Slope	1.15	0.96	1.02	1.19	1.07	1.00	1.07	1.16	1.06	1.03
CI Min	0.97	0.81	0.82	1.01	1.00	0.89	1.04	1.07	1.00	0.96
CI Max	1.32	1.11	1.21	1.36	1.15	1.12	1.10	1.25	1.11	1.10
Intercept	-51	0	9	-74	-44	-3	-0.03	-1.16	-0.03	-0.06
CI Min	-90	-9	-60	-139	-94	-61	-0.04	-1.81	-0.05	-0.43
CI Max	-12	8	79	-10	6	56	-0.02	-0.52	0.00	0.31

Table 3. Validation statistics.

Validation

A total of 23 in-house standards, previously analyzed using ICP-OES and potentiometry, were used to validate UniQuant for EDXRF. Each standard was measured in duplicate. **Figure 1** shows the results obtained with EDXRF (the average of each duplicate) versus those obtained with ICP-OES and potentiometry. **Table 3** gives an overview of the relevant statistical parameters for all elements; standard error of prediction (SEP), correlation coefficient (R^2), slope, intercept, as well as the confidence intervals (CI) of the slope and intercept.

A good statistical agreement is obtained when the fitted straight line has a slope equal to 1 and an intercept equal to 0, considering the confidence limits of both parameters. Slopes and intercepts statistically equal to 1 and 0 respectively, are marked with bold font. **Table 3** shows that for most elements the slope meets this requirement. However, the 95 % confidence intervals for the intercept often don't include 0, indicating that a bias is present. Compared to the results obtained using empirical calibration curves, reported in [XRF-AN41967](#), the graphs in this application note show more scatter and the quality of results is not as good as shown by validation statistics.

However, with the milk-based powder calibration now available in UniQuant software, users can analyze this type of samples without the need for having well characterized in-house standards available. From the user's perspective this is a truly standardless quantification method which provides a reliable albeit semi-quantitative analysis.

Summary

The ARL QUANT'X EDXRF Spectrometer combined with UniQuant standardless fundamental parameters software is a straightforward tool to monitor the elemental composition of milk-based powders. Though the method does not reach the same level of accuracy as an empirical calibration approach based on an extensive set of standards, it provides a (semi-)quantitative analysis without the need for standards.

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