Application note

Analysis of nutrients in milk-based powders by EDXRF

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Introduction

A variety of analytical techniques are used in the food industry to determine the nutritional value of milk-based powders (e.g., infant formula), which consist of milk powder with added nutrients. Examples of nutrients found in these “fortified” milk powders include proteins, fats, carbohydrates, vitamins, and minerals.

Energy-dispersive X-ray fluorescence (EDXRF) is used near the production line to control the elemental content of milk-based powders. A fast and straightforward sample preparation combined with short measurement times help to optimize production processes and minimize turn-around time. In this application note, the elements that are monitored with EDXRF include Na, Mg, P, Cl, K, Ca, Mn, Fe, Cu, and Zn.

Na and Mg remain challenging elements for EDXRF due to their lower sensitivity. Here, we show that substantial improvement in their detection efficiency can be made when using a silicon drift detector (SDD) with a graphene window.

Condition	Voltage (kV)	Current (mA)	Filter	Atmosphere	Live Time (s)	Analytes	ROI Normalizing Background (keV)
Low Za	4	2.00 (Auto)	No Filter	Helium	180	Na, Mg	
Low Zb	8	2.00 (Auto)	C Thick	Helium	60	P, S, K, Ca	4.4 - 6.0
Mid Zb	20	2.00 (Auto)	Ag Medium	Air	180	Mn, Fe, Cu, Zn	13.8 - 17.1

Table 1. Excitation conditions used in this application note.

Instrumentation

The Thermo Scientific™ ARL QUANT'X™ EDXRF Spectrometer is equipped with an end-window X-ray tube of 50 Watt and a Rh or Ag target. The sample was analyzed along a direct excitation geometry with primary beam filters. A total of 9 filters are available to optimize element excitation. A large-area SDD with a graphene window (that is less than 1 µm thick) allows for the detection of all periodic table elements ranging from carbon (Z = 6) up to Am (Z = 95). Until recently, the default SDD window was an 8 µm thick Be window which does not allow for the detection of elements lighter than F (Z = 9).

Sample preparation

For each sample, 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, 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.

For this application, 36 in-house milk-based powder standards were prepared. 13 standards were used for the calibration while the remaining 23 standards were used for the validation.

Measurement conditions

Three excitation conditions were used to measure 10 elements. Table 1 shows the experimental details of each condition. Lighter elements are measured in helium in order to avoid absorption of their low-energy characteristic X-rays by air. Analysis in helium is preferred over vacuum because milk powder particles tend to penetrate the vacuum pump and affect its oil quality.

The total measurement time consisted of 420 s "live time" with 180 s for the two conditions, focusing on light and transition elements respectively. Depending on uncertainty and reproducibility requirements, the measurement time can be reduced, especially for the condition focused on transition elements, where Mn concentrations are near the detection limit. If the determination of Mn is not critical, the measurement time for that condition can be reduced to 60 s.

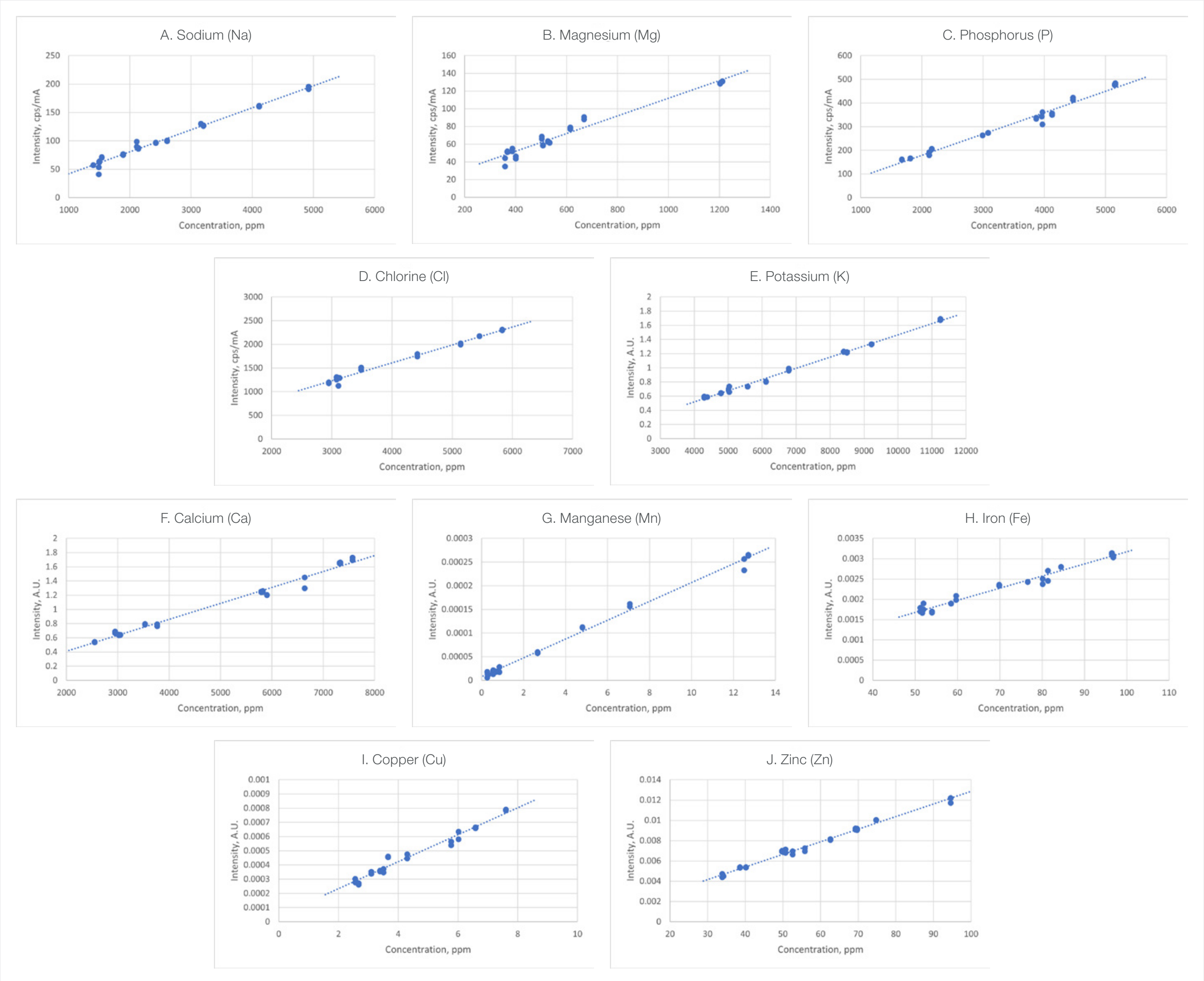

Figure 1. Elemental calibration curves as obtained with EDXRF.

Element	Conc. range (ppm)	RMS (ppm)	R^2
Na	1400 - 4920	157	0.979
Mg	358 - 1212	56	0.961
P	1670 - 5160	172	0.980
Cl	2950 - 5830	126	0.989
K	4290 - 11250	159	0.995
Ca	2550 - 7570	231	0.985
Mn	0.3 - 13	0.4	0.994
Fe	51 - 97	3.0	0.968
Cu	3 - 8	0.3	0.966
Zn	34 - 95	1.9	0.989

Table 2. Elemental concentration ranges and calibration statistics.

Calibration

A total of 13 in-house standards, previously analyzed with inductively coupled plasma optical emission spectroscopy (ICP-OES), are used to set up the calibration. Table 2 shows the concentration ranges for each element; standards are measured in duplicate (both sides of the pellet).

Spectrum fitting is used to derive net elemental peak intensities by correcting for background and spectral overlap.

In some cases, to compensate for the matrix effect caused by variation in the powders' organic composition, and to improve the calibration model, the ratio of the peak intensity to a part of the scattered spectral background was calculated and used instead of merely the element intensity. Such a ratio wasn't applied for all elements as it does not always lead to an improvement. The elements for which this normalization worked well are K, Ca, Mn, Fe, Cu, and Zn.

Figure 1 shows the calibration curves for the different elements. Table 2 also shows the calibration's root mean square error (RMS) and correlation coefficient (R^2) for every element.

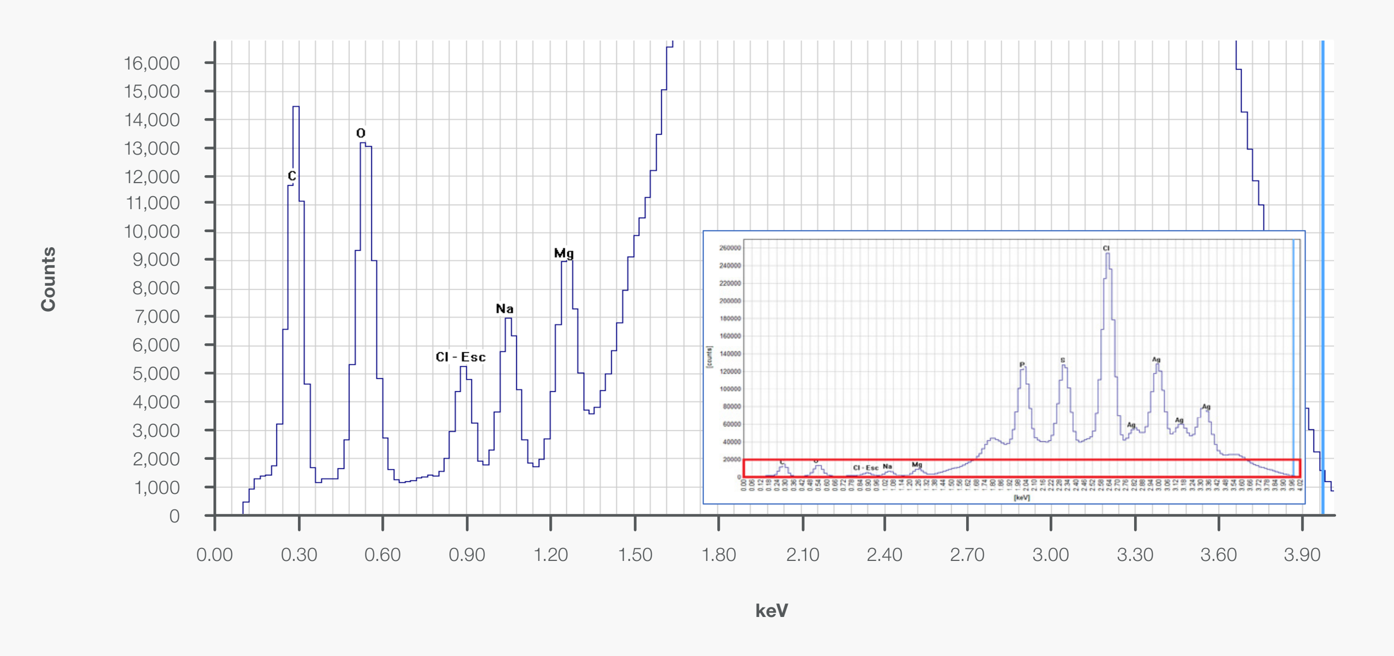

Figure 2. Spectrum of a milk-based powder containing 1,400 ppm Na and 500 ppm Mg, obtained using condition “Low Za” as shown in Table 1 (4 kV, no filter, 180 s live time).

Figure 2 shows the spectrum for one of the standards containing 1,400 ppm Na and 500 ppm Mg, which was collected using excitation condition Low Za (Table 1). The SDD with graphene window has an excellent sensitivity for Na and Mg, generating element peaks with good statistics. In turn, this allows for calibration curves with small RMS values, which was previously impossible with EDXRF.

Validation

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

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. Table 3 shows that for most elements these conditions are met. The elements Ca and Mn do not meet the criteria for both the slope and intercept. In the case of Mn, this is likely a result of the concentration range, which is close to the detection limit of the technique. In the case of Ca, it is suspected that the element isn’t as homogenously distributed throughout the material as the other elements, which lead to discrepancies between the EDXRF and ICP-OES data, since different material volumes were sampled. Alternatively, normalizing to part of the spectral background might not be sufficient to compensate for matrix effects. However, the agreement between EDXRF and ICP-OES data is acceptable, and the slope and intercept values almost meet the requirements.

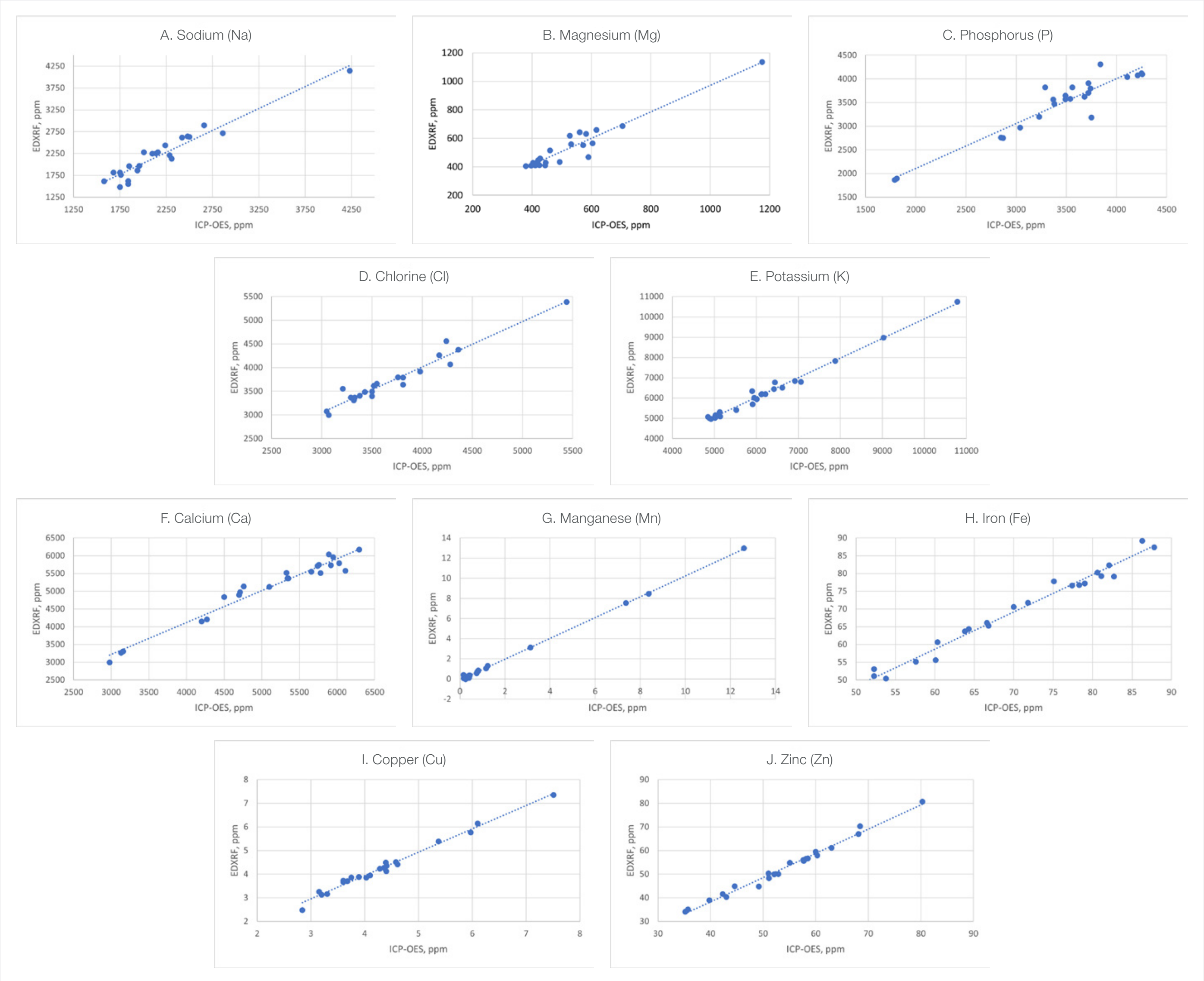

Figure 3. Milk-based powder standards analyzed with EDXRF, as compared to ICP-OES results.

	Na	Mg	P	Cl	K	Ca	Mn	Fe	Cu	Zn
SEP	162	47	221	131	158	203	0.16	2.0	0.14	1.9
R^2	0.921	0.920	0.886	0.945	0.988	0.960	0.999	0.980	0.985	0.987
Slope	1.005	0.929	0.948	0.961	0.967	0.900	1.033	1.047	0.987	1.027
SD of slope	0.064	0.060	0.074	0.053	0.024	0.040	0.008	0.032	0.027	0.026
CI Min	0.872	0.805	0.794	0.849	0.918	0.817	1.016	0.980	0.932	0.973
CI Max	1.139	1.054	1.102	1.073	1.017	0.984	1.050	1.115	1.042	1.081
Intercept	13	42	213	169	227	516	-0.09	-4.1	-0.01	-2.8
SD of intercept	145	33	259	200	152	207	0.03	2.3	0.12	1.4
CI Min	-289	-26	-326	-250	-89	85	-0.15	-8.9	-0.25	-5.8
CI Max	314	110	751	589	543	947	-0.03	0.6	0.24	0.1

Table 3. Validation statistics.

Element	Ref.	Day 1	Day 7	Day 9	Day 12	Day 14	Day 16	Day 19	Day 21	Day 23	Day 26	Day 28
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Na	2160	2202	2156	2164	2143	2200	2196	2195	2190	2183	2188	2205
Mg	417	429	414	413	417	421	418	428	418	425	421	417
P	2850	2678	2715	2691	2698	2727	2703	2719	2688	2688	2678	2704
Cl	3330	3277	3280	3263	3290	3304	3312	3326	3287	3306	3298	3290
K	4920	4871	4860	4869	4863	4902	4898	4882	4877	4887	4884	4891
Ca	4270	4160	4174	4175	4181	4182	4207	4193	4199	4210	4204	4206
Mn	1.2	1.2	1.2	1.2	1.2	1.4	1.5	1.3	1.2	1.6	0.9	1.2
Fe	52.3	54.6	54.2	53.9	54.0	54.5	54.1	54.0	54.1	54.1	53.8	54.0
Cu	4.3	4.2	4.2	4.2	4.3	4.2	4.2	4.3	4.3	4.2	4.2	4.2
Zn	52.1	49.4	48.7	49.2	49.0	49.2	48.6	49.3	48.8	49.2	49.1	49.1

Table 4. Analysis of a validation sample over a period of 28 days.

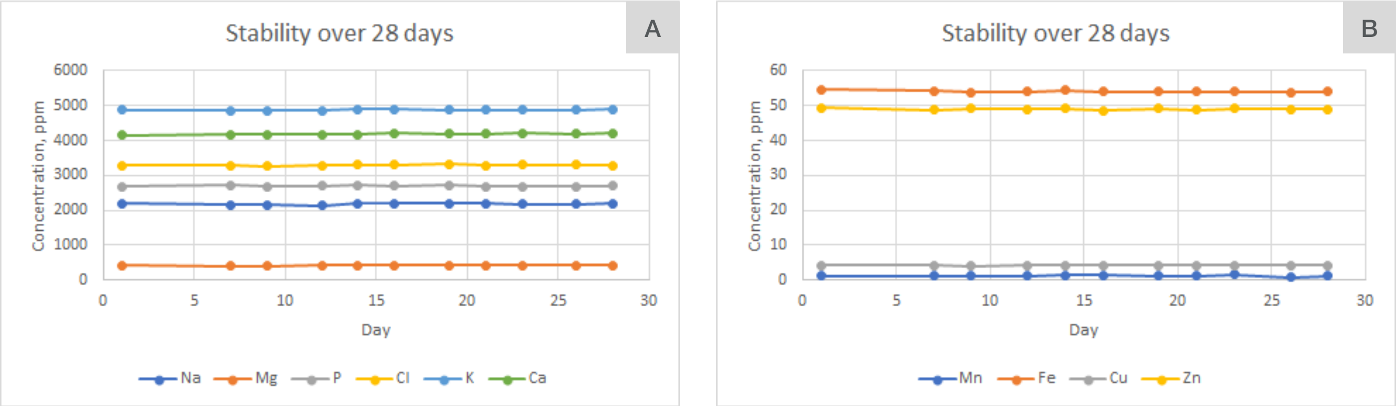

Figure 4. Analysis results over 28 days. A) Elements present at concentrations > 500 ppm. B) Elements present at concentrations < 100 ppm.

Stability over several days

Five validation samples were analyzed regularly over a period of 28 days to assess the stability of EDXRF over time (Table 4). Each reported concentration is the average of replicates. Figure 4 shows the results for one of the validation samples monitored over 28 days. The QUANT'X EDXRF Spectrometer produces stable analysis results, even for elements present at lower ppm concentrations.

Summary

The ARL QUANT'X EDXRF Spectrometer, when equipped with a graphene detector window, is an excellent tool for monitoring the elemental composition of milk-based powders. Light elements such as Na and Mg, which were previously difficult to detect, now show well-defined signals of sufficient intensity to set up reliable calibration curves.

This application note shows how in-house standards are used to set up a calibration, followed by an extensive validation. Any matrix effects are handled by normalizing the elemental peak intensity by part of the spectral background, avoiding any inter-element corrections, and producing simple and reliable calibration models.

The performance of the ARL QUANT'X Spectrometer over a period of 28 days shows good stability over time, reducing the need for drift correction or recalibration.

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