



X-RAY FLUORESCENCE

Selenium Analysis in Total Mixed Rations (TMR) and Compound Feed by XRF

A Method Validation Study

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Abstract

X-ray fluorescence (XRF) spectrometry is widely used in the agri-food and livestock sectors for the quantification of macro- and microelements in animal feed, pet food, and agricultural products (typically Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, and Zn). A recent study conducted by the Laboratorio Analisi Zootecniche in Italy demonstrated excellent analytical performance also for selenium (Se), a trace element typically present at low concentrations and not routinely analyzed by XRF.

To assess analytical performance, XRF data were compared with results obtained using reference techniques (e.g., inductively coupled plasma – mass spectrometry, ICP-MS) for total-mixed ration (TMR) samples, and with certified reference materials (CRMs) for compound feed.

Instrument Calibration and Sample Preparation

The XRF instrument Bruker S2 PUMA, based on energy-dispersive X-ray fluorescence (EDXRF) technology, was calibrated using certified reference materials obtained from interlaboratory proficiency testing schemes organized by Wageningen University & Research (WE-PAL-QUASIMEME, The Netherlands;^[3]) and by Association of American Feed Control Officials (AAFCO, USA;^[4]).

A total of 35 plant-based raw materials and feed samples were included for the calibration of the spectrometer, covering selenium concentrations ranging from 0.06 to 2.6 mg/kg, alongside other macro- and microelements (Table 1). The powder samples were homogenous, dry and fine grained. Eight (8) grams of sample material were pressed at 35 tons for 20 seconds to produce pressed pellets. The pressed pellets were measured in vacuum mode, which is important for light elements sensitivity (e.g. Na, Mg). Selenium K α fluorescence was measured following excitation at 40 keV using a 500 μ m aluminum filter primary beam filter. The resulting calibration showed a correlation coefficient R² of 0.98125 and a standard deviation of 0.102 mg/kg Se (Figure 1). These results indicate a strong linear relationship and robust calibration performance for selenium.

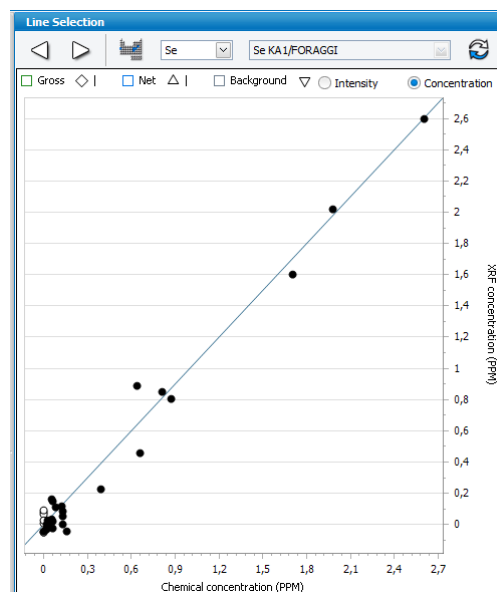
In cooperation with:



Table 1

Compositional range of the WEPAL and AAFCO reference materials used for calibration.

Element	Concentration Range
Na	0.002 – 1.1 wt%
Mg	0.034 – 0.569 wt%
Al	13.6 – 1420 mg/kg
Si	0.0067 – 7.75 wt%
P	0.047 – 1.56 wt%
S	0.057 – 1.47 wt%
Cl	0.083 – 2.105 wt%
K	0.140 – 5.206 wt%
Ca	0.018 – 4.65 wt%
Cr	0.149 – 24 mg/kg
Mn	4.3 – 480.8 mg/kg
Fe	32.09 – 1200 mg/kg
Co	0.008 – 5.06 mg/kg
Ni	0.108 – 13.43 mg/kg
Cu	2 – 221.53 mg/kg
Zn	10.4 – 503.8 mg/kg
As	0.014 – 1.56 mg/kg
Se	0.02 – 2.605 mg/kg
Mo	0.126 – 15.189 mg/kg
Ba	0.09 – 33.66 mg/kg
Pb	0.017 – 3.063 mg/kg

**Figure 1**

Calibration curve for Se KA plant-based raw materials and feed samples as displayed in the spectrometer software, SPECTRA.ELEMENT. Calibration statistics: R² of 0.98125 and a standard deviation of 0.102 mg/kg Se.

Method Validation

Method Validation 1: Total Mixed Rations (Lactation and Dry Period)

The reliability of the method was further evaluated through a validation study involving 11 TMR samples with recipes optimized for the cow's lactation phase and dry period feeding. The TMR samples were dried at 65°C for 16-20 hours and ground by using a mill equipped with a 0.5 mm sieve. The samples were analyzed by using the XRF method described above and in parallel by using ICP-MS as the reference method at the Università Cattolica del Sacro Cuore (Piacenza, Italy; Prof. Gian Maria Beone, Faculty of Agricultural, Food, and Environmental Sciences). For ICP-MS analysis, the dried samples (0.25 g) were subjected to microwave-assisted digestion in a closed-vessel system with infrared temperature control. Digestion was performed using 6 mL of nitric acid (65% w/w) and 2 mL of hydrogen peroxide (30% w/w). The microwave program consisted of a 20 min ramp to 180 °C followed by 15 min at 180 °C, with automatic power adjustment. After cooling, digests were diluted to 50 mL with distilled water and analyzed by ICP-MS.

None of these samples were used for the calibration of the XRF spectrometer.

Results of Method Validation 1 (TMR)

The comparison between XRF and ICP-MS confirms the robustness of the XRF method, particularly within nutritionally relevant concentration ranges:

- **High precision in the critical range (0.5–2.3 mg/kg):** We observed an excellent coefficient of variation (CV) of 9.1%, with a standard deviation of 0.10 mg/kg, ensuring optimal accuracy precisely where monitoring selenium is essential for animal health.

- **Good performance down to very low Se contents (0.2–2.3 mg/kg):** Across the full analyzed range (0.2–2.3 mg/kg), the average CV is 19.6% (standard deviation of 0.14 mg/kg), enabling robust assessment of the Se content even < 0.5 mg/kg.

The dispersion of the XRF data is shown in Figure 2. The actual difference between the XRF and ICP-MS values is listed in Table 2.

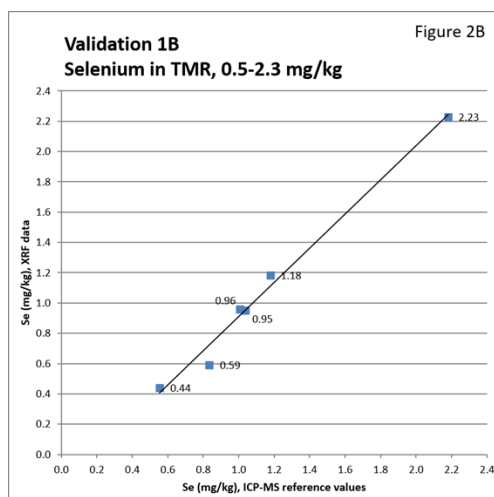
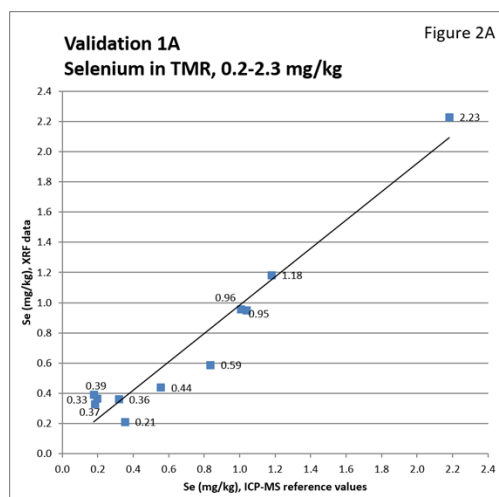


Figure 2

Validation data for Se in TMR. The plots illustrate the good agreement of the concentrations obtained by XRF (values displayed) with the ICP-MS reference values.

Statistics:

Critical concentration range (Fig. 2A, left):

Correlation = 0.972,
Std.Dev. = 0.143 mg/kg,
CV = 19.6%

Wider concentration range (Fig. 2B, right):

Correlation = 0.994,
Std.Dev. = 0.103 mg/kg,
CV = 9.1%.

Table 2

Selenium validation data for TMR

Reference material	Se, mg/kg (ICP-MS)	Se, mg/kg (XRF)	Error Standard	Bias
Ref-1	0.35	0.21	0.14	0.14
Ref-2	0.18	0.33	0.15	-0.15
Ref-3	0.32	0.36	0.04	-0.04
Ref-4	0.19	0.37	0.17	-0.17
Ref-5	0.18	0.39	0.21	-0.21
Ref-6	0.56	0.44	0.12	0.12
Ref-7	0.83	0.59	0.24	0.24
Ref-8	1.04	0.95	0.09	0.09
Ref-9	1.01	0.96	0.05	0.05
Ref-10	1.18	1.18	0.01	-0.01
Ref-11	2.18	2.23	0.05	-0.05
Averages (all)	0.73	0.73	0.11	0.00

Method Validation 2: Compound Feed

A second validation study was conducted by using 16 compound feed samples obtained from the AAFCO interlaboratory proficiency testing program. Nine (9) of these reference materials were already used for the calibration of the XRF spectrometer, whereas 7 additional reference samples were not used for calibration (for details see Figure 3).

Results of Method Validation 2 (Compound Feed)

For compound feed, XRF also demonstrated strong agreement with reference values (Figure 3 and Table 3):

- **Analytical performance for the complete concentration range covered by the method (0.2–2.5 mg/kg Se):** A CV of 12.5% and a standard deviation of 0.12 mg/kg Se proves the robustness of the method for critical levels of Se in compound feed.

Compared to TMR, less variability and higher linearity across the entire Se concentration range was found for compound feed. It is proposed that this is related to the higher compositional and grain size variability of the TMR samples.

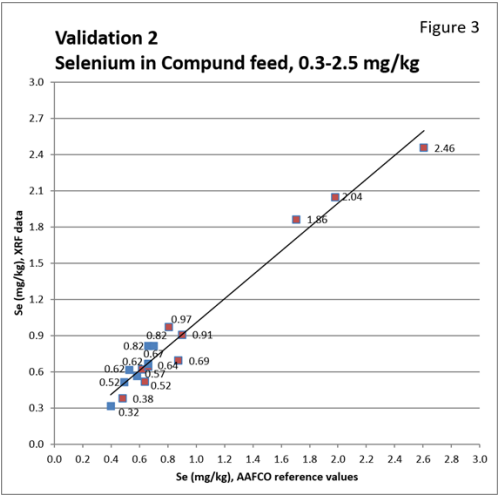


Figure 3
Validation data for Se in compound feed. The plots illustrate the good agreement of the concentrations obtained by XRF (values displayed) with the AAFCO reference values. **BLUE** symbols: Reference materials were not used for calibration; **RED** symbols: Reference materials were used for calibration.

Statistics:
Correlation = 0.984,
Std.Dev. = 0.112,
CV = 12.5%.

Table 3
Selenium validation data for Compound Feed

Reference material	Se, mg/kg (AAFCO)	Se, mg/kg (XRF)	Error Standard	Bias
Ref-1	0.40	0.32	0.08	0.08
Ref-2	0.48	0.38	0.10	0.10
Ref-3	0.49	0.52	0.03	-0.03
Ref-4	0.64	0.52	0.12	0.12
Ref-5	0.58	0.57	0.01	0.01
Ref-6	0.53	0.62	0.09	-0.09
Ref-7	0.62	0.62	0.00	0.00
Ref-8	0.66	0.64	0.02	0.02
Ref-9	0.66	0.67	0.01	-0.01
Ref-10	0.88	0.69	0.18	0.18
Ref-11	0.66	0.82	0.16	-0.16
Ref-12	0.70	0.82	0.12	-0.12
Ref-13	0.81	0.97	0.16	-0.16
Ref-14	1.71	1.86	0.15	-0.15
Ref-15	1.98	2.04	0.06	-0.06
Ref-16	2.61	2.46	0.15	0.15
Average	0.90	0.91	0.09	-0.01

Conclusions

In this study, the performance of a method (or calibration) for the analysis of critical levels of Se in animal feed materials by EDXRF was validated.

A total of 27 TMR and compound feed samples were used for the validation. For both materials, the analytical error increased as selenium concentration decreased, as expected for trace-level analysis. Improved correlation was observed for concentrations above approximately 0.3–0.5 mg/kg, with CV values in the range of 10–12%. Across the full analytical range (0.2–2.5 mg/kg), the average CV remained below approximately 20%.

These findings confirm that EDXRF is a highly reliable and rapid screening tool for accurate selenium determination in feed matrices, offering a significant advantage over the longer turnaround times associated with traditional wet chemical methods.

Authors

⁽¹⁾ Dr. Marco Mancinelli, biologist, serves as Technical Director of an R&D laboratory, with expertise in NIR and XRF technologies and a background in biochemistry. Together with Eugenio Mancinelli, he is a partner at the Laboratorio Analisi Zootecniche in Gonzaga (Mantua, Italy). For over 30 years, he has been engaged in chemical and physical analysis for the agri-livestock sector.

⁽²⁾ Dr. Adrian Fiege, geoscientist, is a product manager for XRF at Bruker in Germany. After his PhD (University of Hannover) and postdoc (University of Michigan), he was an EPMA and FT-NIR lab manager at the Museum of Natural History in New York, US. Adrian joined Bruker in 2019 and became an expert for inorganic analysis in the food and animal feed industry.

Further information

^[1] Details about WEPAL-QUASIMEME: <https://www.wur.nl/en/research/products-services/proficiency-testing-wepal-quasimeme>

^[2] Details about AAFCO: Proficiency Testing Program | AAFCO

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