



ACRYRED Interlaboratory Ring Test 2026

Determination of Acrylamide in Low-Acrylamide Cereal Products

Final Report

Reported by

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Executive Summary

The second interlaboratory ring test organised within **COST Action CA21149 ACRYRED** focused on the determination of acrylamide in low-acrylamide cereal products. Compared with the first ACRYRED ring test conducted in 2025, this exercise addressed more analytically challenging concentration levels relevant to current discussions on benchmark and maximum levels for cereal-based foods, including products intended for infants and young children.

A total of **18 laboratories received the test materials**, and **13 laboratories (72%) from 10 countries** submitted results suitable for statistical evaluation. Participants were requested to analyse the samples within a predefined two-week period (18-29 May 2026), and **8 laboratories completed the analyses within this interval**.

Two homogenised cereal matrices were distributed: **SK-2026-1 (organic popped rice cake)** and **SK-2026-2 (organic extruded corn snack)**. Robust statistical evaluation according to ISO 13528 established assigned values of **107.4 µg/kg** and **49.6 µg/kg**, respectively.

Reported concentrations ranged from **22 to 280 µg/kg** for SK-2026-1 and from **23 to 168 µg/kg** for SK-2026-2. Despite these relatively wide ranges, most laboratories achieved satisfactory performance. For SK-2026-1, 11 laboratories obtained satisfactory results, one generated a warning signal and one an unsatisfactory result. For SK-2026-2, 12 laboratories achieved satisfactory performance, and one laboratory produced an unsatisfactory result.

A separate evaluation of accredited laboratories demonstrated particularly strong performance. **No accredited laboratory generated an unsatisfactory result**, confirming the suitability of validated LC-MS/MS methodologies for routine acrylamide determination.

The most important finding relates to the extruded corn snack sample, for which the assigned value (**49.6 µg/kg**) was very close to the proposed regulatory limit of **50 µg/kg** for processed cereal-based foods for infants and young children. The results demonstrate that analytical variability and measurement uncertainty may significantly influence compliance assessment at such low concentration levels.

Compared with the first ACRYRED ring test, participation increased markedly and the exercise addressed substantially lower acrylamide concentrations. The results demonstrate improved harmonisation of analytical methodologies across the ACRYRED network and highlight the importance of continued proficiency testing, harmonised quality assurance procedures and careful consideration of measurement uncertainty when establishing and enforcing very low benchmark or maximum levels for acrylamide in cereal-based foods and foods intended for infants and young children.

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OBJECTIVES

Following the successful completion of the first ACRYRED interlaboratory comparison in 2025, a second ring test was organised within COST Action CA21149 ACRYRED.

While the first exercise focused primarily on matrices containing moderate to elevated concentrations of acrylamide, the 2026 round specifically addressed the analytical challenges associated with low-acrylamide cereal products. These products are particularly relevant in the context of current discussions on benchmark and maximum levels for cereal-based foods intended for infants and young children.

The objectives of the exercise were to:

- evaluate laboratory performance in the determination of acrylamide at low concentration levels,
- assess interlaboratory comparability of analytical methods,
- compare the performance of accredited and non-accredited laboratories,
- identify potential sources of analytical variability,
- provide evidence relevant to future regulatory discussions regarding low acrylamide levels.

Test Material

Two homogenised cereal matrices were selected:

SK-2026-1: Organic popped rice cake

SK-2026-2: Organic extruded corn snack

Both products were expected to contain relatively low concentrations of acrylamide.

Samples were ground to powder at 5000 rpm for 15 s using a Grindomix GM200 (Retsch, Germany).

Each sample was packed in a sealed plastic bag (15 g) and stored at ambient temperature.

Participating Laboratories

A total of **18 laboratories** received the test materials, either during the ACRYRED Training School 15-17 April 2026 in Plovdiv (Bulgaria) or by postal delivery. Together with the test materials, all participants received written instructions describing the objectives of the exercise, storage conditions, reporting requirements and the testing schedule. Participants were requested to store the samples at ambient temperature (18-22 °C) and to perform the analyses within the predefined testing period from **18 to 29 May 2026**.

Most participating laboratories complied with the testing schedule defined in the accompanying instructions. Of the **13 laboratories providing results**, **8 laboratories completed the analyses within this specified interval**, while one laboratory analysed the samples at earlier dates and four laboratories at later dates due to logistical or operational reasons. The reported analysis dates covered the period

from April to September 2026, providing an opportunity to assess the stability of the test materials over an extended period.

A total of 13 laboratories from 10 countries submitted results suitable for statistical evaluation. Each laboratory was assigned a unique code to maintain anonymity. Participation by the laboratories was voluntary.

List of participating countries:

Cyprus, Czech Republic, Germany, Greece, Montenegro, Portugal, Serbia, Slovakia, Spain, United Kingdom



Participating laboratories represented Northern, Western, Central, Southern and South-Eastern Europe, demonstrating the broad geographical coverage of the ACRYRED network.

Analytical Methods

Each participant was requested to provide details of the analytical method:

- technique for determining acrylamide,
- expression of acrylamide in $\mu\text{g}/\text{kg}$ in whole numbers – no decimals,
- indication of % yield (if relevant),
- indication of standard deviation (if relevant),
- use of internal standard (if yes, which one),
- use of reference material (if yes, which one),
- indication of LOD and LOQ,

- whether the method is validated (yes/no)
- whether the method is accredited (yes/no)
- date of analysis

Details of analytical methods (techniques, internal standard, reference material, LOD/LOQ, validation/accreditation) are listed in Table 1.

Table 1. Overview of analytical method

Lab Code	Accreditation	Analytical Technique	Internal Standard	Reference Material	LOD (µg/kg)	LOQ (µg/kg)
L01	No	LC-MS/MS	¹³ C-acrylamide	n.r.	n.r.	n.r.
L02	No	HS-SPME GC/MS	No	No	7.6	25.6
L03	No	GC-MS	d3-acrylamide	No	5	10
L04	No	LC-MS/MS	¹³ C-acrylamide	No	3.5	15
L05	Yes	LC-MS/MS	d3-acrylamide	Coffee substitute	3	10
L06	Yes	LC-MS/MS	d3-acrylamide	No	5.3	16
L07	No	LC-MS/MS	d3-acrylamide	No	10	20
L08	Yes	LC-MS/MS	not reported	n.r.	n.r.	n.r.
L09	Yes	GC-MS/MS	d3-acrylamide	No	3	10
L10	No	LC-MS/MS	d3-acrylamide	Biscuits	10	20
L11	No	LC-MS/MS	No	FAPAS Biscuits	10	20
L12	Yes	LC-MS/MS	¹³ C-acrylamide	No	7.5	25
L13	Yes	UHPLC-MS/MS	¹³ C-acrylamide	No	3	10

n.r. – not reported

All participating laboratories applied chromatographic methods coupled with mass spectrometric detection, namely LC-MS/MS, UHPLC-MS/MS, GC-MS or GC-MS/MS. Stable isotope-labelled acrylamide internal standards (¹³C3-acrylamide, d3-acrylamide) were used by almost all participants, reflecting current best practice for acrylamide determination. Only two laboratories did not report the use of an isotopically labelled internal standard.

Most reported limits of detection ranged between 3 and 10 µg/kg, while limits of quantification ranged between 10 and 25.6 µg/kg. These values were well below the acrylamide concentrations observed in the test materials and therefore did not limit analytical performance.

Only three laboratories reported the use of reference materials during routine quality control.

The results demonstrate a high degree of methodological harmonisation within the ACRYRED network. Consequently, the observed interlaboratory variability is unlikely to originate from differences in analytical principle, but rather from factors such as sample preparation, extraction efficiency, calibration strategy, data processing and routine quality assurance practices.

RESULTS

Reported Values of Acrylamide

Within the specified period, the participating laboratories submitted their results by email, together with a description of the method and parameters used for the determination. Reported values are listed in Table 2 and Figures 1 and 2.

Table 2. Reported acrylamide levels ($\mu\text{g}/\text{kg}$) and uncertainty (U %) of the samples SK-2026-1 and SK-2026-2

Lab	Analysis Date	SK-2026-1 ($\mu\text{g}/\text{kg}$)	U (%)	SK-2026-2 ($\mu\text{g}/\text{kg}$)	U (%)
L01	01.06.2026	116	2	43	2
L02	29.7.2026	22	10	56	29
L03	28.05.2026	92	7	45	14
L04	19.05.2026	147	4	66	6
L05	21.05.2026	70	20	23	20
L06	26.05.2026	121	11	46	11
L07	26.06.2026	108	3	62	1
L08	13.06.2026	96	22	29	9
L09	27.05.2026	117	15	76	15
L10	21.05.2026	132	6	50	10
L11	25.05.2026	280	8	168	5
L12	21.05.2026	62	15	31	15
L13	22.04.–04.05.2026	121	20	39	36

Reported concentrations ranged from **22 to 280 $\mu\text{g}/\text{kg}$** for SK-2026-1 and from **23 to 168 $\mu\text{g}/\text{kg}$** for SK-2026-2. Despite these relatively wide ranges, most laboratories reported values within **70-147 $\mu\text{g}/\text{kg}$** for SK-2026-1 and **29-76 $\mu\text{g}/\text{kg}$** for SK-2026-2, indicating good overall agreement within the main participant group.

It is noteworthy that eight of the thirteen participating laboratories complied with the prescribed two-week testing period (17–30 May 2026). Despite some laboratories performing the analyses out of this interval, **no systematic relationship was observed between the analysis date and the reported acrylamide concentration**. This finding indicates **good stability** of both homogenised test materials during storage and transport and supports their suitability for future interlaboratory comparison exercises.

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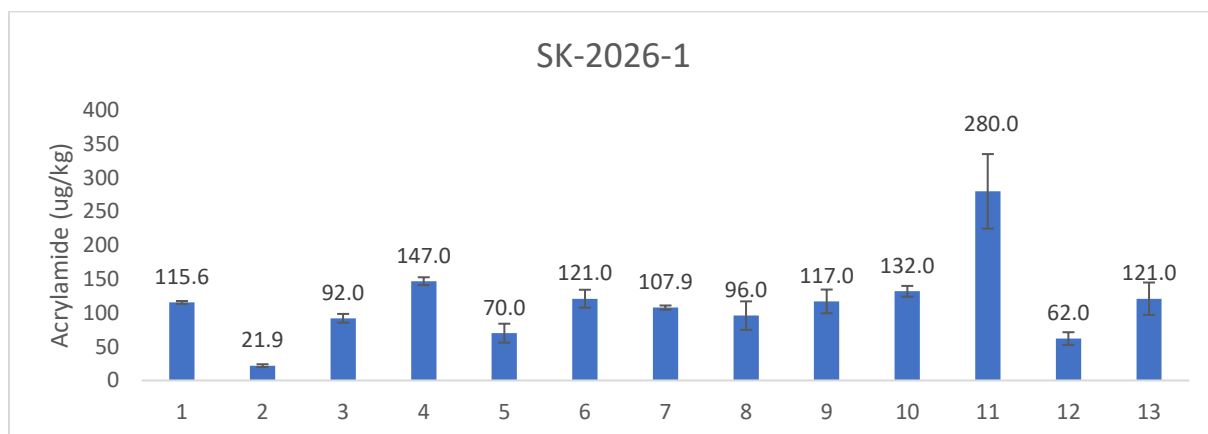


Fig. 1 Reported acrylamide levels (µg/kg) of the samples SK-2026-1

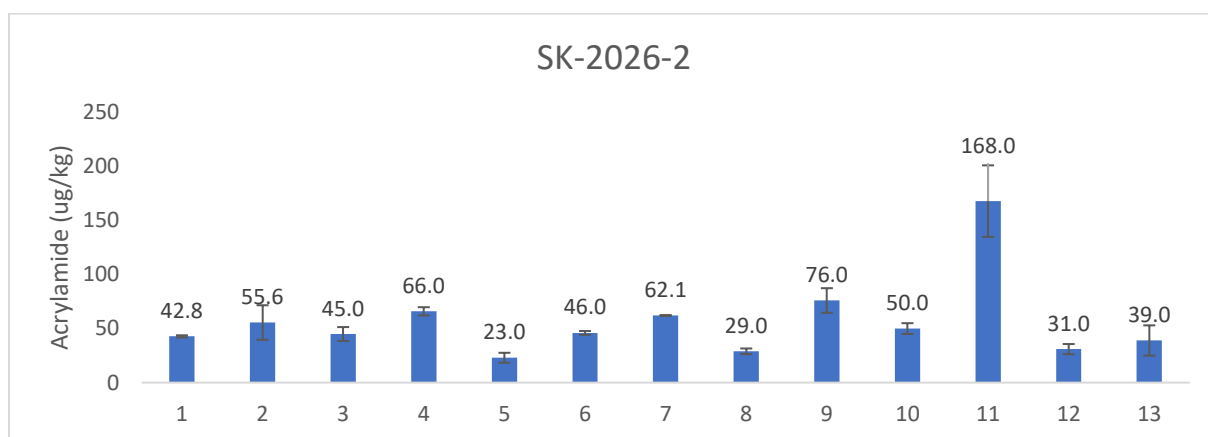


Fig. 2 Reported acrylamide levels (µg/kg) of the samples SK-2026-2

The two test materials represented distinctly different, yet analytically relevant, concentration levels.

Robustness of the Results

The statistical evaluation demonstrated that the assigned values were robust despite the presence of extreme observations in the dataset.

Table 3. Classical and Robust Statistical Parameters for Samples SK-2026-1 and SK-2026-2

Parameter	SK-2026-1	SK-2026-2
Number of results	13	13
Arithmetic mean (µg/kg)	114.1	56.4
Median (µg/kg)	115.6	46.0
Standard deviation (µg/kg)	59.9	36.8
Relative standard deviation (CV, %)	52.5	64.1
Minimum (µg/kg)	21.9	23.0
Maximum (µg/kg)	280.0	168.0

Parameter	SK-2026-1	SK-2026-2
Robust assigned value (µg/kg)	107.4	49.6
Robust standard deviation (µg/kg)	36.8	20.0

The robust assigned value for SK-2026-1 (organic popped rice cake) was 107.4 µg/kg, while the assigned value for SK-2026-2 (organic extruded corn snack) was 49.6 µg/kg. The concentration in the rice cake sample was therefore approximately twice that observed in the corn snack sample.

The popped rice cake sample showed a relatively wide distribution of reported results, ranging from 22 to 280 µg/kg. Most laboratories, however, clustered around the assigned value, with eleven of the thirteen participants reporting concentrations between approximately 70 and 147 µg/kg. The extreme value reported by Laboratory L11 had a substantial influence on the arithmetic mean but only a limited effect on the robust assigned value.

The extruded corn snack sample represented a particularly challenging matrix because its assigned value (50 µg/kg) lies very close to concentration levels currently discussed for certain categories of cereal-based infant foods. Although most laboratories reported concentrations between 29 and 76 µg/kg, the overall range extended from 23 to 168 µg/kg. This demonstrates that determination of acrylamide at low concentrations remains considerably more demanding than at moderate or elevated levels.

A notable observation is that the same laboratory (L11) reported substantially higher concentrations for both samples (280 µg/kg and 168 µg/kg, respectively). The consistency of this deviation suggests a systematic analytical bias rather than random measurement variability. Conversely, most laboratories reported values that were remarkably consistent across both matrices, indicating good method performance and comparability.

From a practical and regulatory perspective, the most important finding concerns the corn snack sample. The assigned value of 49.6 µg/kg is very close to a proposed maximum level 50 µg/kg for infant and young children foods. For this sample, some laboratories reported results below 50 µg/kg, whereas others reported values substantially above this threshold. Consequently, the same product could potentially be classified as either compliant or non-compliant depending on the laboratory performing the analysis.

Reported Measurement Uncertainties

Reported expanded measurement uncertainties varied considerably among participating laboratories, ranging from 2% to 22% for Sample SK-2026-1 and from 1% to 36% for Sample SK-2026-2. The median uncertainties were approximately 10% and 11%, respectively, indicating that most laboratories considered their routine analytical uncertainty to be around one tenth of the reported value.

The results confirm that analytical uncertainty becomes increasingly important when assessing products with acrylamide concentrations close to 50 µg/kg. For example, a concentration of 50 µg/kg

reported with a 20% expanded uncertainty corresponds to an interval of approximately 40–60 µg/kg, potentially affecting compliance decisions if regulatory limits are established at this level.

Tab 4. Statistical summary of measurement uncertainties

Statistic	SK-2026-1 (%)	SK-2026-2 (%)
Minimum	2	1
Maximum	22	36
Mean	11	13
Median	10	11

Effect of Outlying Results

For both samples, the arithmetic mean was noticeably influenced by extreme values.

For sample SK-2026-1, the arithmetic mean was 114.1 µg/kg, whereas the robust assigned value was 107.4 µg/kg. The difference of approximately 7 µg/kg was caused mainly by one laboratory reporting a concentration of 280 µg/kg, more than twice the consensus value.

A similar effect was observed for SK-2026-2, where the arithmetic mean (56.4 µg/kg) exceeded the robust assigned value (50.0 µg/kg) because of an extreme result of 168 µg/kg.

The use of robust statistics therefore prevented a small number of extreme observations from disproportionately influencing the assigned values and performance assessment.

Relative Variability

The classical coefficients of variation were relatively high:

- 52.5% for SK-2026-1
- 65.2% for SK-2026-2

These values reflect the analytical difficulty of determining acrylamide at low concentration levels and the sensitivity of low-level measurements to differences in sample preparation, calibration and quantification procedures.

However, the robust standard deviations obtained after application of ISO 13528 Algorithm A were substantially lower than the classical standard deviations, demonstrating that most laboratories formed a well-defined consensus group and that the observed variability was driven primarily by a limited number of extreme results.

Robustness of Regulatory Conclusions

Importantly, the robust assigned values remained stable despite the presence of outliers. This indicates that the consensus concentrations of approximately:

- **107.4 µg/kg for the popped rice cake sample,**
- **49.6 µg/kg for the extruded corn snack sample,**

can be considered reliable estimates of the concentrations present in the test materials.

Thus, the conclusions regarding laboratory performance, interlaboratory comparability and potential implications for future benchmark or maximum levels are not dependent on the inclusion or exclusion of individual laboratories

The robust statistical approach prescribed by ISO 13528 proved highly effective for evaluation of low-acrylamide matrices. Although individual extreme results were observed, most participating laboratories demonstrated good agreement, resulting in stable assigned values and reliable performance assessment. The robustness of the consensus values supports their use for evaluating analytical capabilities relevant to future acrylamide limits in cereal-based foods and baby foods.

Evaluation of z-Scores

The **z-score** is a standardized statistical indicator used in proficiency testing to assess how closely an individual laboratory result agrees with the assigned value. It expresses the deviation of a reported result from the consensus value in units of the standard deviation used for proficiency assessment. A z-score close to zero indicates excellent agreement with the assigned value, while larger positive or negative values indicate increasing deviation from the consensus result. According to ISO 13528, results with $|z| \leq 2$ are considered satisfactory, values between 2 and 3 represent a warning signal, and values ≥ 3 indicate unsatisfactory performance.

z-scores were calculated according to ISO 13528:

$$z = \frac{x_i - x_{pt}}{\sigma_{pt}}$$

where:

- x_i = participant result,
- x_{pt} = assigned value,
- σ_{pt} = standard deviation for proficiency assessment.

Classification:

z-score	Interpretation
$ z \leq 2$	Satisfactory
$2 < z < 3$	Warning signal
$ z \geq 3$	Unsatisfactory

The z-score evaluation demonstrated good overall agreement among participating laboratories despite the low acrylamide concentrations present in both matrices.

For **SK-2026-1**, 11 laboratories achieved satisfactory performance ($|z| \leq 2$), one laboratory generated a warning signal (L02; $z = -2.33$) due to a substantially lower reported value than the consensus result, and one laboratory produced an unsatisfactory result (L11; $z = 4.70$).

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For **SK-2026-2**, 12 laboratories achieved satisfactory performance. No warning signals were observed. One laboratory (L11) produced an unsatisfactory result ($z = 5.92$).

The similarity of z-scores obtained by L11 for both matrices suggests a systematic positive bias rather than random analytical variability.

Table 5. z-Scores for SK-2026-1 and SK-2026-2

Lab	Result (µg/kg)	z-score	Evaluation	Result (µg/kg)	z-score	Evaluation
L01	116	0.22	Satisfactory	43	-0.34	Satisfactory
L02	22	-2.33	Warning	56	0.30	Satisfactory
L03	92	-0.42	Satisfactory	45	-0.23	Satisfactory
L04	147	1.08	Satisfactory	66	0.82	Satisfactory
L05	70	-1.02	Satisfactory	23	-1.33	Satisfactory
L06	121	0.37	Satisfactory	46	-0.18	Satisfactory
L07	108	0.01	Satisfactory	62	0.62	Satisfactory
L08	96	-0.31	Satisfactory	29	-1.03	Satisfactory
L09	117	0.26	Satisfactory	76	1.32	Satisfactory
L10	132	0.67	Satisfactory	50	0.02	Satisfactory
L11	280	4.70	Unsatisfactory	168	5.92	Unsatisfactory
L12	62	-1.24	Satisfactory	31	-0.93	Satisfactory
L13	121	0.37	Satisfactory	39	-0.53	Satisfactory

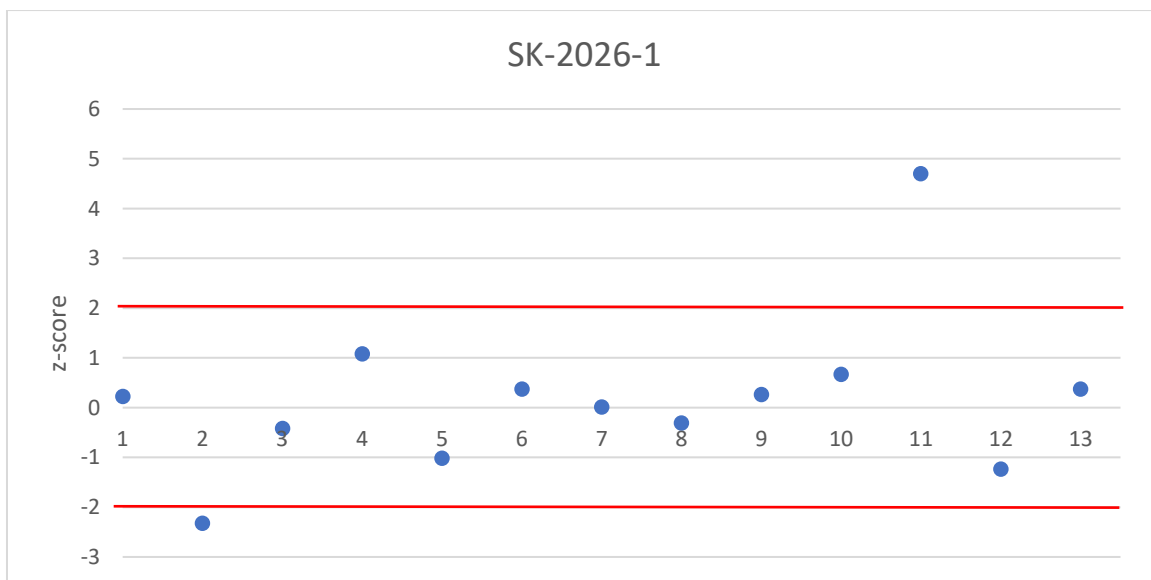


Fig. 3 z-Scores obtained by participating laboratories for sample SK-2026-1

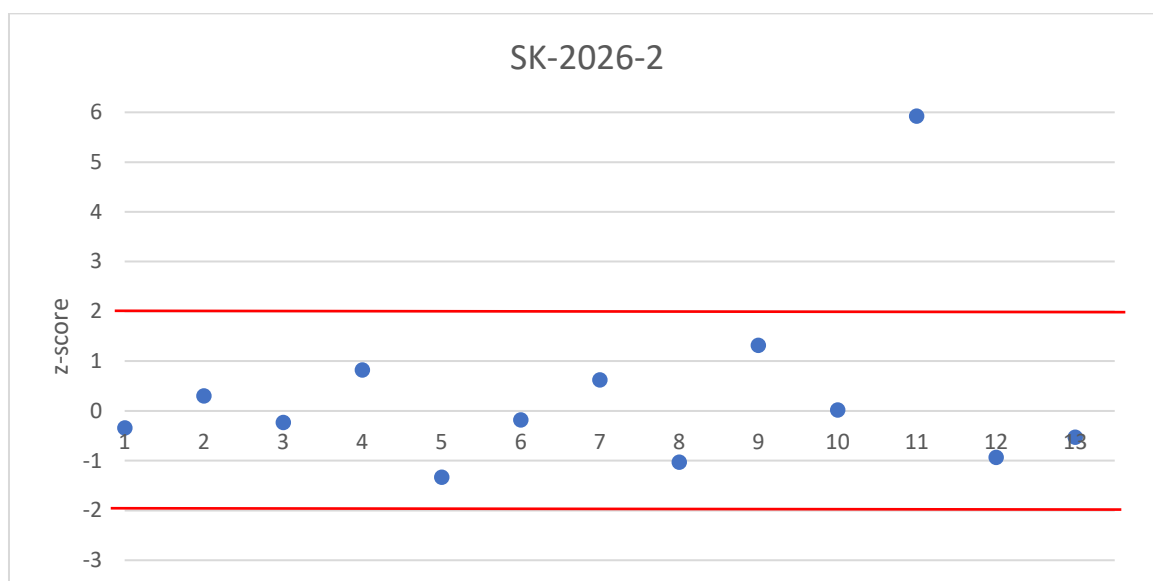


Fig. 4 z-Scores obtained by participating laboratories for sample SK-2026-2

Consistency of Laboratory Performance

An important observation is that the same laboratory (L11) obtained markedly elevated results for both test materials, resulting in unsatisfactory z-scores in both cases. This consistency indicates the presence of a systematic analytical bias, potentially related to calibration, quantification procedures, recovery correction, or matrix effects.

Conversely, all accredited laboratories achieved satisfactory performance for both samples, confirming the high level of analytical harmonisation within the accredited subgroup.

The z-score distributions also demonstrate that most participating laboratories clustered closely around the assigned values, supporting the robustness of the consensus concentrations determined according to ISO 13528.

Performance of Accredited Laboratories

Accredited laboratories reported considerably **higher measurement uncertainties** (mean 17.2% and 17.9% for SK-2026-1 and SK-2026-2, respectively) than non-accredited laboratories (5.6% and 9.1%). This should not be interpreted as poorer analytical performance. On the contrary, accredited laboratories demonstrated **the best interlaboratory agreement and no unsatisfactory z-scores**. The higher reported uncertainties most likely reflect more comprehensive uncertainty estimation procedures required by accredited quality systems, whereas lower reported uncertainties may indicate a less conservative approach to uncertainty evaluation.

The accredited laboratories demonstrated acceptable performance according to proficiency-testing criteria; however, the spread of reported results remained considerable. For the low-acrylamide sample, results ranged from 23 to 76 µg/kg, indicating that even among accredited laboratories substantial analytical variability persists. These findings suggest that current analytical methodologies

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can detect low acrylamide concentrations, but further harmonisation may be necessary if regulatory limits are set close to these concentration levels.

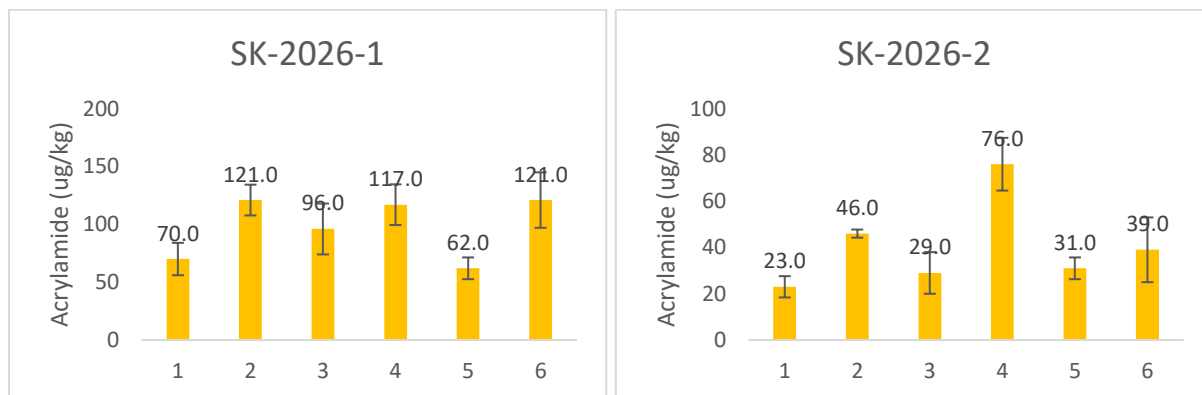


Fig. 5 Reported acrylamide levels (µg/kg) of the samples SK-2026-1 and SK-2026-2 from accredited laboratories

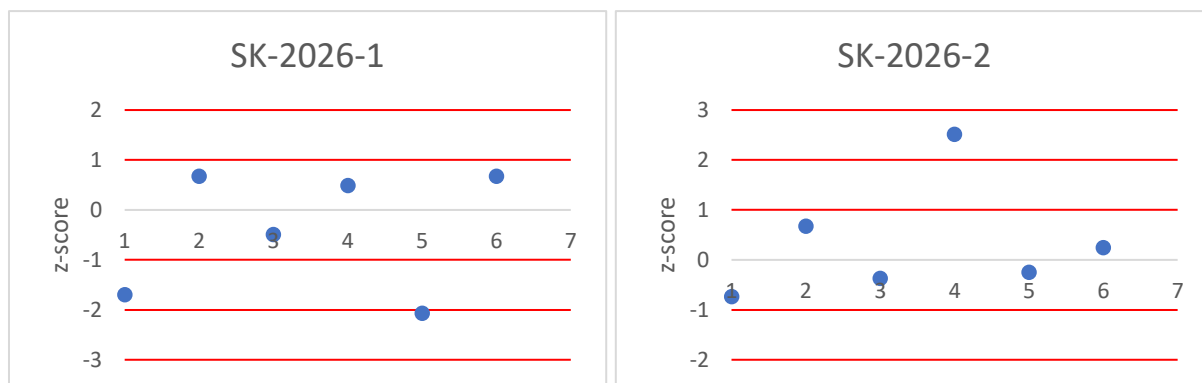


Fig. 6 z-Scores obtained by accredited laboratories for samples SK-2026-1 and SK-2026-2

To conclude the performance of accredited laboratories:

- Almost all accredited laboratories achieved **satisfactory performance** for both samples.
- One accredited laboratory generated a warning signal.
- No accredited laboratory generated an unsatisfactory result.

CONCLUSIONS

Overall, the ring test demonstrated that reliable determination of acrylamide at concentrations around 50 µg/kg is achievable but still associated with appreciable interlaboratory variability. These findings highlight **the importance of considering measurement uncertainty and harmonised analytical procedures** when establishing and enforcing very low maximum levels for acrylamide in cereal-based foods and baby foods.

The results therefore indicate that:

- concentrations around **50 µg/kg** represent a challenging analytical region where interlaboratory variability and measurement uncertainty may significantly influence compliance assessment;
- concentrations around **100 µg/kg** can generally be determined with greater consistency and lower risk of misclassification;
- concentrations of **150 µg/kg** and above are likely to be classified with high confidence by the majority of laboratories.

The second ACRYRED ring test confirmed that reliable determination of acrylamide at concentrations close to 50 µg/kg is achievable by most participating laboratories, particularly those operating under accredited quality systems. However, the observed interlaboratory variability and reported measurement uncertainties indicate that compliance assessment near very low regulatory limits requires careful interpretation. The exercise demonstrated a growing level of harmonisation within the ACRYRED network and provides valuable evidence for future discussions on benchmark and maximum levels for acrylamide in cereal-based foods and foods intended for infants and young children.

Summary of the First and Second ACRYRED Ring Tests

The two ACRYRED interlaboratory comparisons demonstrated a progressive improvement in the harmonisation of acrylamide determination across the network. While the first ring test focused on matrices containing moderate to higher acrylamide concentrations, the second round deliberately addressed the more demanding task of quantifying acrylamide in low-level cereal products relevant to infant and young child nutrition. Participation increased from 7 laboratories in 2025 to **13 laboratories from 10 countries** in 2026, substantially strengthening the statistical robustness of the exercise. The results confirmed that modern chromatographic methods coupled with mass spectrometric detection provide reliable determination of acrylamide across a wide concentration range, particularly when implemented within accredited quality systems.

Across both rounds, most participants achieved satisfactory performance, and the robust statistical evaluation successfully minimized the influence of occasional outlying results. The second round further demonstrated that analytical variability becomes increasingly important at concentrations around **50 µg/kg**, where measurement uncertainty and interlaboratory differences may affect compliance assessment. Nevertheless, all accredited laboratories achieved satisfactory performance, confirming that validated and quality-assured methodologies are fit for purpose even at low acrylamide concentrations.

Overall, the ACRYRED ring tests have provided valuable evidence supporting the comparability and reliability of acrylamide measurements within the network while highlighting the importance of continued proficiency testing, harmonised quality assurance procedures, and careful consideration of measurement uncertainty when setting and enforcing future benchmark or maximum levels for acrylamide in cereal-based foods and baby foods.

REFERENCES

ISO/IEC 17043:2023 – *Conformity assessment — General requirements for proficiency testing*

ISO 13528:2022 – *Statistical methods for use in proficiency testing by interlaboratory comparisons*