Chemical Tests: How to understand your measurement method and your result

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Regional Workshop on QA/QC of Flour Fortification
Dr. Philip Randall
Outline

1. Measurement methods
2. Validation of methods
3. Measurement uncertainty
4. Case study A: vitamin A fortified flour
5. Case study B: iron fortified flour
Measurement methods for micronutrients in food: CODEX

Standard 234 – 1999 contain method amendments adopted 2014

1. Fluorometry
2. Colorimetry
3. Spectrophotometry
4. Micro- and rat-bioassay
5. Rat bioassay
6. HPLC (added 2011)

Accessed 2015
Recommended methods for specific applications

IRON
- AAS (Atomic absorption spectroscopy)
- ICP-OES (Inductively coupled plasma atomic emission spectroscopy)
- Colorimetric assay

FOLIC ACID
- Microbiological assay
- Immunoassay
- Optical Biosensors

VITAMIN A
- Colorimetric assay
- Fluorometry
- HPLC (high performance liquid chromatography)

IODINE
- ICP-OES
- Colorimetric assay
- Titration
Each measurement method must be validated for a specific type of food and the type of micronutrient

<table>
<thead>
<tr>
<th>IRON</th>
<th>VITAMIN A</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaFeEDTA</td>
<td>Vitamin A palmitate</td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>Vitamin A acetate</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>Vitamin A propionate</td>
</tr>
<tr>
<td>Electrolytic iron</td>
<td>Encapsulated vitamin A</td>
</tr>
</tbody>
</table>

- Intrinsic iron
- Electrolytic iron
- Intrinsic iron
Validation assesses the accuracy of the measurement
International Standards Organization definition of accuracy

According to ISO 5725-1, **Accuracy** consists of Trueness and Precision

**Trueness** - proximity of measurement results to the true value

**Precision** - repeatability or reproducibility of the measurement
PRECISION
Multiple factors influence the precision of measurement

- the sample itself
- the analyst
- the instrument
- the environment
Validation protocol example for precision assessment

- Fortified flour sample
- Diluted flour sample

- 1st analyst
- 2nd analyst
- 3rd analyst

- 1st instrument
- 2nd instrument
- 3rd instrument

- 1st day
- 2nd day
- 3rd day

Intra–assay variation
Inter–person variation
Inter–day variation
Inter–device variation
Calculation of precision based on results of repeated measurements

\[
\frac{\text{STANDARD DEVIATION}}{\text{MEAN}} \times 100\% = \text{Coefficient of variation (CV) in } \% \\
\]

Coefficient of Variation with 95% confidence level = 1.96 x CV

Example: \((4 \text{ ppm}/40 \text{ ppm}) \times 100\% = 10\%\)  
95% confidence = \(1.96 \times 10\% = 19.6\%\)
AACC ring trial of 14 USA laboratories with 7 flour samples shows significant variation

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Minimum CV x 2</th>
<th>Maximum CV x 2</th>
<th>Mean CV x 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>12%</td>
<td>54%</td>
<td>22%</td>
</tr>
<tr>
<td>Zinc</td>
<td>9%</td>
<td>16%</td>
<td>13%</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>63%</td>
<td>370%</td>
<td>141%</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>30%</td>
<td>82%</td>
<td>45%</td>
</tr>
</tbody>
</table>

http://www.aaccnet.org/checksample/
Validation protocol example for trueness assessment

- Flour sample

\[ \text{Iron} \]

\[ 10 \text{ mg/kg} \] \[ 20 \text{ mg/kg} \] \[ 100 \text{ mg/kg} \]
Calculation of trueness is based on the recovery of added analyte.

\[
\text{Trueness} = \left( \frac{\text{MEASURED AMOUNT}}{\text{ADDED AMOUNT}} \right) \times 100\% = \text{Trueness in } \%
\]

Trueness is expressed in terms of bias: how far is the result from the true value in %?

Example: \((38 \text{ ppm}/40 \text{ ppm}) \times 100\% = 95\% \)

Bias: \(100\% - 95\% = 5\% \)
ACCURACY
Accuracy = precision + trueness

- **Precision** is expressed in terms of coefficient of variation (CV) in % at 95% confidence level
- **Trueness** is expressed in terms of bias: how far is the result from the true value in %
- **Accuracy** is expressed in terms of Measurement uncertainty, that combines CV and Bias.
Calculation of measurement uncertainty to express the accuracy

BIAS + (1.96 x CV) = Measurement Uncertainty, %

Example: 5% + (1.96 x 10%) = 24.6%
How to use Measurement Uncertainty to present your analytical result

Example:
- Measurement uncertainty was assessed for testing iron in fortified flour using one specific method (i.e. AAS, iCheck Iron, spectrophotometry) and was calculated to be 25%.
- A sample of fortified flour with unknown concentration was measured and the result is 35 ppm (mg/kg).
- The standard is set at minimum 40 ppm.
- Does this sample comply? The answer is „YES“!
- With the measurement uncertainty of 25% the result has a range that is following:
  - 35 ppm ±25%
  - 35 ± 9 ppm
  - 26 – 44 ppm
- With measurement uncertainty you are 95% certain that your result is true.
Sampling: Representative sample

Variation between single small samples can be as high as ±100%.

Pool/Combine multiple single samples in one composite sample

Control Sample
Conditions to be met for proper quality control

1. Measurement Uncertainty is Known
2. Sample is Representative
3. Standard is Set
4. Permitted Tolerance is Set
Case Study A: Vitamin A in flour

1. Measurement Uncertainty
   ±20%

2. Representative Sample
   ✓

3. Standard
   2 ppm

4. Permitted Tolerance
   +50% -20%
It is important not to base a decision only on one result. Collect the samples of the same origin (same brand) during a period of time to get an understanding what is the trend: is it consistent or is it highly variable?

Permitted tolerance is your definition what is acceptable difference of measured result from the target.
Case Study A: Vitamin A in flour

- PPM (MG/KG) vs. MONTH
- Tolerance +50%
- Standard Tolerance -20%
Case Study A: Vitamin A in flour

It is important not to base a decision only on one measured sample. Collect the samples of the same origin during a 

samples of the same origin during a
Case Study B: Iron in flour

1. Measurement Uncertainty ±25%
2. Representative Sample ✔
3. Standard 20 ppm
4. Permitted Tolerance ±20%
Case Study B: Iron in flour
Additional factor is Intrinsic iron

1. Self-rising white flour: 10-20 ppm
2. Bread flour: 20-40 ppm
3. Brown flour: 40-60 ppm
Scenario 1: Self-rising flour with 20 ppm natural iron; 20 ppm added iron
Scenario 1: Self-rising flour with 20 ppm natural iron; 20 ppm added iron
Scenario 1: Self-rising flour with 20 ppm natural iron; 20 ppm added iron
Scenario 2: Bread flour with 30 ppm natural iron; 20 ppm added iron
Scenario 2: Bread flour with 30 ppm natural iron; 20 ppm added iron
Case Study B: Iron in flour
Additional factor is Intrinsic iron

1. Self-rising white flour: 10-20 ppm
2. White bread flour: 20-40 ppm
3. Brown bread flour: 40-60 ppm

Legal definition of different flour types and implications for total iron standard and tolerance limits!!!!
If the analysis results are repeatedly at the upper/lower limits of the permitted tolerance, the food business operator must adopt more effective in-house control and make the necessary changes in the production process or the labelling.
If the indicated nutrient contents of the foodstuff deviate repeatedly from the set tolerance limits, taking the measurement uncertainty of the analysis method into account, the foodstuff is not acceptable and may not be kept for sale.
Optimal Situation
THANK YOU!

Dr. Anna Zhenchuk
Technical Director
E-mail: anna.zhenchuk@bioanalyt.com
www.bioanalyt.com