

Vitamin A in baby porridge containing fruit and milled cereals

Measurand				Uncertainty estimation		
Analyte & technique	Unit	Sector & matrix	Sampling target	Purpose	Design	Statistics
Vitamin A (as retinol) HPLC	µg/100 g in powder	Food baby porridge-powder containing fruit	Produced batch	Total measurement uncertainty	Empirical duplicate method	One-way ANOVA

1 Scope

The scope is to estimate the measurement uncertainty and contributions from sampling and analyses. The estimates are based on samples from one type of baby porridge - taken from 10 different batches - using a sampling protocol collecting duplicate samples from each batch.

2 Scenario and sampling target

In the production of baby (infant) porridge, the vitamin A (retinol) is added as a premix (together with vitamin D and vitamin C). The premix is a minor ingredient. All ingredients are mixed thoroughly before distribution into packages. Earlier analysis indicated a bigger variation in analytical result between packages than expected. A measurement uncertainty of 20 - 30 % would be considered acceptable. The question was raised if the variation mainly is due to analytical uncertainty or to sampling uncertainty. One of the theories suggests that the vitamin is locally unevenly distributed within the package, and therefore will give bigger analytical uncertainty if the test portion is too small e.g. 3-5 g¹. One possible explanation of the heterogeneity is that the vitamin premix aggregates in small hot-spots, due to electrostatic interactions with the fruit particles in the porridge powder. The producers recommend a test portion size of 40 – 50 g whenever analysing vitamin A, D and C in baby porridge powder.

In order to compare the measured vitamin A concentration against declared values and European regulatory thresholds, an estimation of measurement uncertainty is desirable. To determine random component of the measurement uncertainty, an empirical approach using the Duplicate Method (see UfS-Guide 9.4.2) is chosen. To estimate the systematic component a comparison with a reference values is made

3 Sampling protocol

Normally a spot sampling approach - one sample (one package) of a batch - is used as screening when comparing the content with declared values and legal limits.

Validation - In this study two samples are collected from each of 10 different batches of one type of baby porridge powder. Each sample is equal to one package of approximately 400 g powder.

Quality Control - Quality control (QC) of sampling from different types of baby porridge is

¹ EN-12823-1 “Foodstuffs – determination of vitamin A by HPLC” indicates a test sample of approximately 2 – 10 g

done by collecting two samples from each of 8 batches of different types of baby porridges. All the types of porridges contain fruit in addition to milled cereals.

To ensure the quality in each package of the product at the time of “best before date” of the porridge powder, the producer wraps the product in an air tight and light protecting bag. It is therefore assumed the degradation of the vitamin A is negligible during normal self life. The sampling for the validation was performed at the producer. For QC, the samples were purchased partly at the producers, partly at the retailer. When the samples were collected at the retailer – care was taken to collect the two samples (of one product) at different retailers in addition to assure the samples had the same batch marking.

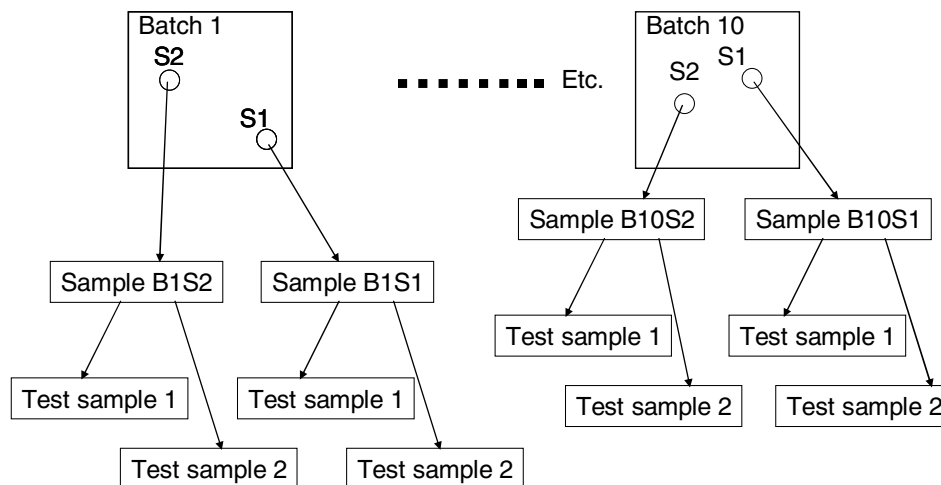
4 Study design – Empirical approach

An empirical (‘top down’) approach – duplicate method was selected to provide estimates of the random component of sampling uncertainty. The validation is performed on one type of baby porridge containing fruit and milled cereals. In the sampling for the QC different products of baby porridge (all containing fruit and milled cereals) are tested to see if the estimate for measurement uncertainty from the validation study is appropriate for different types of baby porridges containing fruit and milled cereals.

4.1 Validation

Samples are collected on line (just after the filling operation of packages) at random time. Two samples (2 packages, each of approximately 400 g) are collected from each of 10 production units (batches) of one type of baby porridge powder.

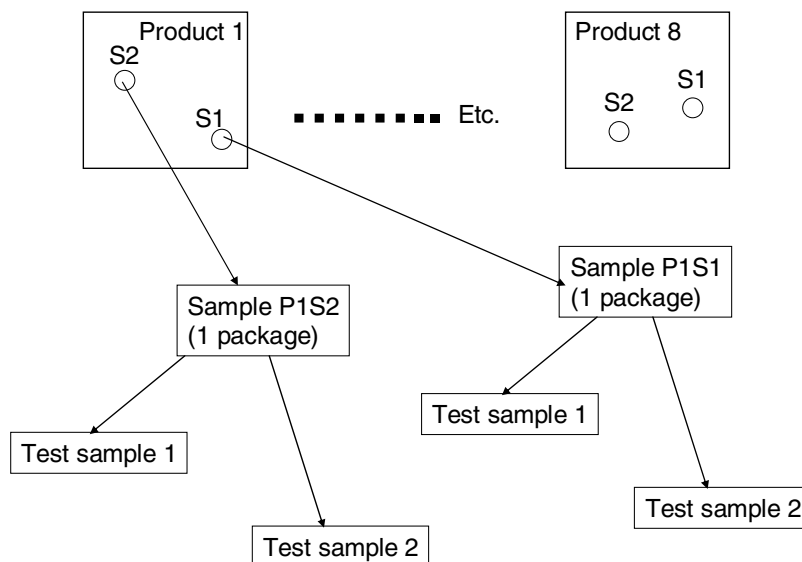
Figur 1 – Sampling for validation. Two samples are taken from each of 10 production units/batches of the same type of baby porridge



4.2 Quality control

For quality control (QC) two samples are collected from one batch of each of 8 different types of baby porridges, containing fruit and milled cereals. The porridges are products from three different producers. The samples (except for two types of porridges) were provided by two of the producers. The rest was bought at the retailer.

Figur 2 - Sampling for QC. Two samples are taken from one batch of each of 8 different types of baby porridge



5 Sampling and analysis in the laboratory

The analytical work is done by “The National Institute of Nutrition and Seafood Research” (NIFES). The laboratory is accredited according to EN ISO/IEC 17025

The laboratory participates in Laboratory Proficiency Tests (FAPAS and Bipea) with good results (in the period 2000 – 2005, $|Z\text{-score}| < 1$). The method is validated using a CRM. Data concerning the laboratory performance is given in the table below.

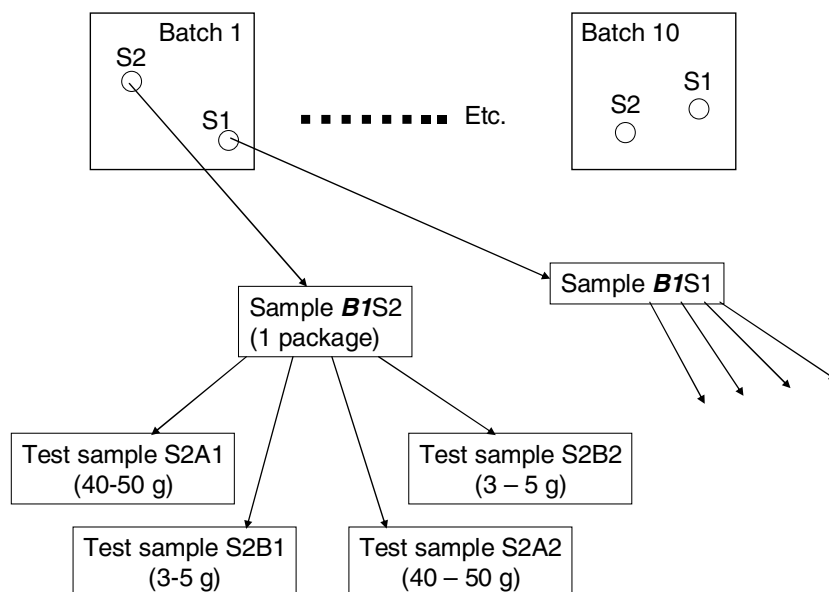
Table 1 - Methods and performance data from quality control - laboratory analyses

Parameters	Vitamin A - determined as retinol
Method	HPLC – normal phase column - UV-detection
Repeatability	2RSD (%) = 6
Within-reproducibility	2RSD(%) = 8
Measurement uncertainty	14 % (95 % confidence interval)
Recovery	<ul style="list-style-type: none"> ➤ Standard addition, in lab: 90 – 110 % ➤ Based on laboratory PTs (in period 1999 – 2005), different matrixes: 88 – 113 %, mean recovery 100,5 %
Limit of Quantification (LOQ)	0.14 mg/kg
CRM used	NIST 2383 – baby food (mixed food composite)
➤ CRM - certified level	0.80 ±0.15 mg/kg (95% confidence interval)
➤ CRM - analysed value	0.77 ±0.14 mg/kg (n=28, 95% confidence interval)

5.1 Secondary sampling

A mechanical sample divider (Retsch) is used to split the samples. From each of the primary samples, 4 test samples are collected; two portions of approximately 3-5 g and two portions of approximately 40 – 50 g.

Figur 3 - Splitting of the primary sample to make 4 test samples



5.2 Analyses

The analytical method is based on EN-12823-1 (Foodstuffs – Determination of vitamin A by HPLC – Part 1: Measurement of all-trans-retinol and 13-cis-retinol). Retinol is saponified by using ethanolic potassium hydroxide containing antioxidants. Vitamin A is extracted by using hexane. Analyse is performed by using High Performance Liquid Chromatography (HPLC), with UV detector.

In the validation, for each of the primary samples, two analyses are performed on test samples of 40 – 50 g and two analyses on test samples of 3 – 5 g. In the QC two analyses are performed on test samples of 40 – 50 g. On each test sample one analytical run is performed (no duplicates).

6 Information from the producer

Data for estimating the “true value” of vitamin A in baby porridge are provided by the producer (Nestlé) of the product chosen for the validation, se table 2.

Table 2 - Product data provided by the producer

Product	Oatmeal porridge with bananas and apricots (Nestlé)
Weight of batch, including premix (1 batch = 2 mixing containers)	1092 kg

Weight of added vitamin-premix in batch	1.228 kg
Vitamin A in premix (data from the Certificate of Analysis)	9016 IU/g = 2705 µg/g (retinol).
Vitamin A added to the batch	304 µg/100 g (retinol)
Vitamin A in ingredients according to the product specification	45 µg/100 g (retinol)
Estimated “true value” of Vitamin A	349 µg/100 g (retinol)
Vitamin A declared as	Retinol - (Sum of trans- and cis- Retinol)

7 Results

Test sample 40 g – baby porridge

Table 3 - Validation data - from the same product, results given in µg/100 g powder

Batch	S1A1	S1A2	S2A1	S2A2
B1	402	325	361	351
B2	382	319	349	362
B3	332	291	397	348
B4	280	278	358	321
B5	370	409	378	460
B6	344	318	381	392
B7	297	333	341	315
B8	336	320	292	306
B9	372	353	332	337
B10	407	361	322	382

S1 and S2: Primary samples from sampling location 1 and 2 of one production batch

A1 and A2: Analyses of duplicate test samples of a primary sample S

Analysed mean value (test sample 40 g): 348 µg/100 g

Test sample 4 g – baby porridge

Table 4 - Validation data – same product, results given in µg/100 g powder

Batch	S1B1	S1B2	S2B1	S2B2
B1	400	491	323	355
B2	413	159	392	434
B3	315	391	252	454
B4	223	220	357	469
B5	462	343	262	293
B6	353	265	305	456
B7	298	234	152	323
B8	425	263	417	353
B9	622	189	291	272
B10	292	397	142	568

S1 and S2: Primary samples from sampling location 1 and 2 of one production batch

B1 and B2: Analyses of duplicate test samples of a primary sample S

Analysed mean value (test sample 4 g): 341 µg/100 g

7.1 Calculations

The ANOVA calculation can be done by using available tools in Excel, Minitab, SPSS etc. In this study the calculations are done in an excel spreadsheet and the details are shown in section 10 – ANOVA calculations.

Calculation of uncertainty of analyses, one-way ANOVA, test sample 40 g

Table 5 - Results from ANOVA calculations – uncertainty of analyses - sum of squares of differences, within groups (SS-Error). For details see section 10

SS_{E-Anal} (µg/100g) ²	Degree of freedom (df)	Variance $= SS_{E-Anal}/df$ (µg/100g) ²	Standard deviation, SD_{anal} $= \sqrt{(SS_{E-Anal})/df}$ (µg/100g)	Relative standard deviation $RSD_{anal}(\%)$ $= (SD / \bar{X}_a) * 100\%$
16595	20	829.75	28.805	8.28

Calculation of uncertainty of sampling, one-way ANOVA, test sample 40 g

Table 6 – Results from ANOVA calculations – uncertainty of sampling - sum of squares of differences. For details see section 10.

SS_S (µg/100g) ²	Degree of freedom (df)	Variance $V_{Samp} =$ $(SS_S/df_S - SSE_{Anal}/df_A)/2$ (µg/100g) ²	Standard deviation, SD_{samp} $= \sqrt{V_{Samp}}$ (µg/100g)	Relative standard deviation $RSD_{samp}(\%)$ $= (SD / \bar{X}_s) * 100\%$
14231	10	296.7	17.22	4.95

Calculation of measurement uncertainty – 40 g test sample

The $RSD(\%)$ value from the ANOVA calculation can be used as an estimate of the standard measurement uncertainty $u(\%)$. The analytical laboratory has estimated the analytical standard uncertainty to be 7 % which is lower than the random analytical component for this sample type - 8.28 %. The higher value of these two is used in the calculations. Combining the RSD values from table 5 and 6 with equation (1), the results can be written as in table 7.

$$u_{meas} = \sqrt{(u_{smp})^2 + (u_{anal})^2} \quad (\text{eq.1})$$

Table 7 Measurement uncertainty – 40 g test sample

Measurement uncertainty, ANOVA calculations – 40 g test samples			
	Sampling	Analytical	Total
Uncertainty u (%)	4.95	8.28	9.7
Expanded uncertainty U (%) = 2*u With a coverage factor of 2 (i.e. 95 % confidence)	9.9	17	20

Calculation of uncertainty of analyses, one-way ANOVA, test sample 4g

The same calculations are used as for test sample size of 40 g (see table 14)

Table 8 - Results from ANOVA calculations – uncertainty of analyses, 4 g test sample - sum of squares of differences, within groups (SS-Error)

SS_E ($\mu\text{g}/100\text{g}$) ²	Degree of freedom (df) ($N*2-N$)=20	Variance = SS_E/df ($\mu\text{g}/100\text{g}$) ²	Standard deviation, SD_{anal} = $\sqrt{SS_E/df}$ ($\mu\text{g}/100\text{g}$)	Relative standard deviation $RSD_{anal}(\%)$ = $(SD / \bar{X}_a)*100\%$
312206.5	20	15610.325	124.9413	36.6800

Calculation of uncertainty of sampling, one-way ANOVA, test sample 4 g

The same calculations are used as for test sample size of 40 g (table 15)

Table 9 - Results from ANOVA calculations – uncertainty of sampling, 4 g test sample - sum of squares of differences

SS_S ($\mu\text{g}/100\text{g}$) ²	Degree of freedom (df)	Variance $V_{Samp} = (SS_S/df_S - SSE_{Anal}/df_A)/2$ ($\mu\text{g}/100\text{g}$) ²	Standard deviation, SD_{samp} = $\sqrt{V_{Samp}}$ ($\mu\text{g}/100\text{g}$)	Relative standard deviation $RSD_{samp}(\%)$ = $(SD / \bar{X}_s)*100\%$
102860.25	10	-2662.15	$\sqrt{-2662.15}$ No calculation is possible!	Can't be calculated

The calculated estimate of SD_{anal} and SD_{samp} has an uncertainty. The negative value of V_{Samp} indicates that the SD_{samp} is small compared to the uncertainty of the calculated value of SD_{anal} . However, in this case SD_{anal} and SD_{samp} can be estimated by using robust ANOVA. (Robust ANOVA gives the following estimates: $u_{Samp}(\%)=6.9\%$ and $u_{Anal}(\%)=30\%$ - but robust ANOVA calculation is not included in this example).

As the sampling is identical for the experiments with 40 g test sample and 5 g test sample (and the uncertainty of sampling therefore should be the same), a $RSD_{samp}(\%) = 5\%$ (≈ 4.95 see table 6) is used as an estimate.

Calculation of measurement uncertainty – 4 g test sample

Using the calculated RSD (%) value in table 8 and 6 as an estimate of the measurement uncertainty and combining with equation (1) the results can be written as follows (table 10):

Table 10 - Measurement uncertainty – 4 g test sample

Measurement uncertainty, ANOVA calculations – 4 g test samples			
	*1 Sampling	Analytical	Measurement
Uncertainty u (%)	5	36.7	37
Expanded uncertainty U (%) = $2*u$ With a coverage factor of 2 (i.e. 95 % confidence)	10	73.4	74

* i The $u(\%)$ value is derived from calculations using 40 g test samples

7.2 Effect of the size of test sample on measurement uncertainty

The baby porridge powder looks homogeneous, and therefore a low measurement uncertainty (u) is expected. However analyses of the powder indicated in fact a surprisingly large u when using a test sample size of 4 g (the CEN-standard EN-12823-1 indicates a test sample size of approximately 2 – 10 g). The producers recommended using a test sample size of 40 – 50 g.

The validation tests gave the following results

Table 11 – Comparing measurement uncertainty- test samples of 40 g and 4 g

Test sample size	Measurement uncertainty (u_{meas})	Expanded measurements uncertainty U_{meas}
40 g test sample	9.7 %	20 %
4 g test sample	37 %	74 %

It can be concluded that $u_{40\text{g}} \ll u_{4\text{g}}$. A U_{meas} of approximately 20 % is acceptable while a U_{meas} of 74 % is considered to be too high, taking into account the matrix and production conditions of this type of product..

Therefore it can be concluded that a test sample weight of 4 g is not “fit for purpose“ when analysing vitamin A (retinol) in baby porridge powder containing milled cereals and fruit. A test sample size of 40 – 50 g is recommended. This also supports the theory that the vitamin is unevenly distributed in the product, possible as local “hot spots” due to electrostatic interactions.

7.3 Quality control

According to UFS guide – section 13.2.2, the principal tool in QC is replication. This is minimally executed by taking two samples from each target by a complete (and suitably randomised) duplication of the sampling protocol. There is only a need to analyse the sample once and the difference between the results $d = |x_1 - x_2|$ is calculated. In this study each sample was analysed twice, but the comparisons was made between one analyses of each sample (double set)

In the quality control study, test portions of 40 g were used. According to declarations, the products contains different amount of vitamin A

Table 12: Quality control data - test portion 40 g – different products

Product	Producer	Porridge powder ingredients	S1A1	S1A2	S2A1	S2A2
P1	1	Oat, rice and pear	322	319	350	375
P2	1	Oat, rye, rice and pear	332	317	358	393
P3	1	Wheat, banana and apple	443	430	461	388
P4	1	Wheat and apple	318	383	390	334
P5	2	Oat, rice and banana	252	219	265	227
P6	2	Wheat and apple	274	239	233	217
P7	2	Oat, rice and apple	206	225	198	195
P8		Wheat, spelt, oat and apple	392	335	375	416

3	(organic product)				
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S1 and S2: Primary samples (laboratory samples) from sampling location 1 and 2 of one batch from each product

A1 and A2: Analyses on two test samples form each laboratory sample.

Quality control - calculation and control chart

If the validated uncertainties of sampling and analysis are u_{samp} and u_{anal} respectively, the expectation of the difference between duplicated results (different samples from same batch) would be zero with an uncertainty of $\sqrt{2(u_{\text{samp}}^2 + u_{\text{anal}}^2)}$. A one-sided control chart with a action limit $L_a = 3 * \sqrt{2(u_{\text{samp}}^2 + u_{\text{anal}}^2)}$ represents control. In the case of baby porridge (40 g test sample) the following calculations can be made:

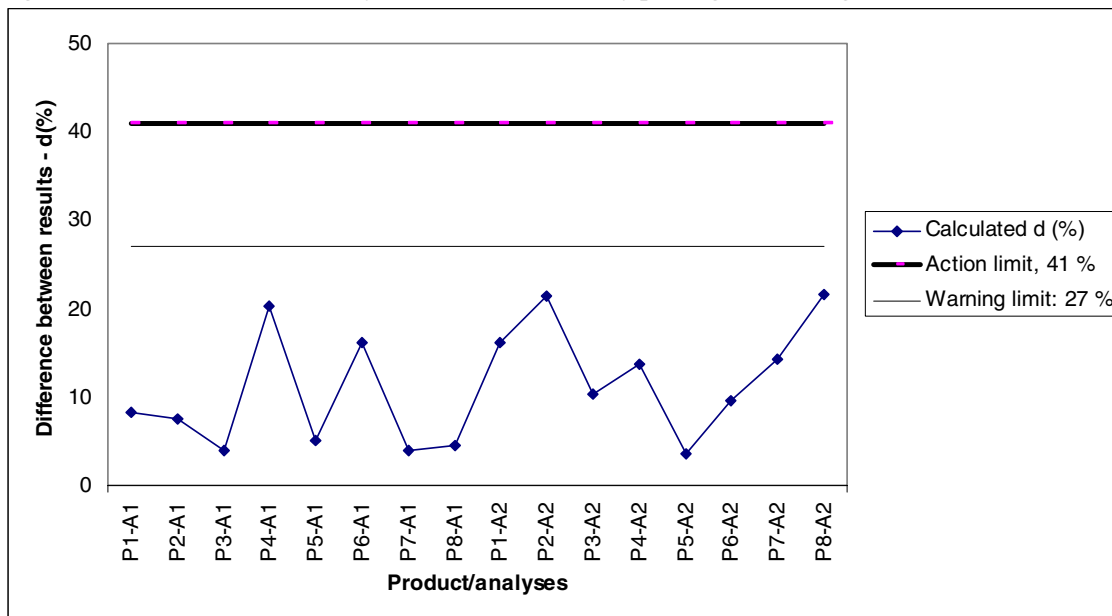
$$L_a = 3 * \sqrt{2 * (4.95^2 + 8.3^2)} \% = 41 \%$$

Table 13 – Quality control: Calculation of differences –d and d(%) - between samples from a batch

Product	Analyses	Sample S1 X_{S1}	Sample S2 X_{S2}	$d = x_{S1} - x_{S2} $	\bar{x}	$d(\%) = (d / \bar{x}) * 100\%$
P1	A1	322	350	28	336	8
P2		332	358	26	345	8
P3		443	461	18	452	4
P4		318	390	72	354	20
P5		252	265	13	259	5
P6		274	233	41	254	16
P7		206	198	8	202	4
P8		392	375	17	384	4
P1	A2	319	375	56	347	16
P2		317	393	76	355	21
P3		430	388	42	409	10
P4		383	334	49	359	14
P5		219	227	8	223	4
P6		239	217	22	228	10
P7		225	195	30	210	14
P8		335	416	81	376	22

The calculated d (%) in table 13 can be compared directly with the action limit, or the results can be presented in a control chart, see figure 4.

Figure 4 – Control chart, QC analyses of vitamin A in baby porridge containing cereals and fruits



The control chart in figure 4 shows that when collecting duplicated samples from same batch, the difference between analytical results d (%) is smaller than the action limit L_a . All the calculated difference are in fact smaller than a warning limit of $2 * \sqrt{2 * (4.95^2 + 8.3^2)} \% = 27$ %.

The measurement uncertainty determined in the validation step is therefore considered suitable for the QC of the sampling of baby porridge containing milled cereals and fruit.

If normal procedure is to analyse one sample from each batch, it is recommended that duplicate samples are collected from the same batch at least in one of ten of the sampled batches.

Measurement uncertainty

Sampling uncertainty

Calculations from the validation study gave an expanded sampling uncertainty $U_{\text{sam}} (\%) = 10$ % (40 g test sample – see table 7). The calculated uncertainty does not include contributions to the uncertainty due to “between protocol” and “between samplers” differences.

Analytical uncertainty

Calculation from the validation study gave an expanded measurement uncertainty of analyses (U_{anal}) of 17% - 40 g test sample. The laboratory reports their own estimation of the analytical uncertainty (see table 1): $2 * \text{RSD}_{\text{inlab}} (\%) = 14\%$. $2 * \text{RSD}_{\text{inlab}} (\%)$ is used as an estimate of U_{Anal} in the laboratory. The U_{anal} found in the validation study was at the same level but still a little bigger than the U_{Anal} reported by the laboratory.

The CRM used is 2383 (NIST) – baby food composite. The CRM is a mix of different foods of plant and animal origins – and the uncertainty found when analysing the CRM might not be identical with the one found when analysing baby porridge powder. Laboratory data for the CRM 2383 is included in the table below.

CRM 2383	Mean value mg/kg	U (%) _{95%}	Laboratory bias (%)
Certified	0.80 ± 0.15	18.8	-
Analysed	0.77 ± 0.14	18.2	-3.75

The measurement uncertainty and the bias determined for the CRM can be included in the analytical measurement uncertainty (see UFS Guide: Example 2) – but as the matrix in the validation study is different from the one in the CRM used, we chose not to include it in this study.

Total measurement uncertainty

Calculations from the validation study gave an expanded measurement uncertainty $U_{\text{meas}}(\%) = 20\%$ (40 g test sample – see table 7).

Systematic bias:

The laboratory reports a recovery of normally 90 – 110 %. Recovery based on laboratory PTs 1999-2005: 88 – 113 %. The results for the PT indicate no (or very small) systematic bias. Analyses of CRM 2383 in the laboratory gives a mean analysed value of 96.3 % of the certified value – which indicates a small bias (-3.7 %). As the matrix of the CRM “baby food composite” is different to the baby porridge, and the analytical method includes an extraction, the bias determined when analysing the CRM might not be representative for the analyses of baby porridge.

In the validation study, the mean value of retinol was determined to be 348 µg/100 g (when using a test sample of 40 g). According to data provided by the producer (see table 2), the “true value” for retinol was calculated to be 349 µg/100 g porridge powder. This gives a recovery of 99.7 % of the “true value”. This gives an indication that the systematic error due to sampling and analyses is small and might be neglectable when analysing baby porridge-powder containing milled cereals and fruits – on the condition that a test sample of 40 – 50 g is used.

8 Comments

When a test sample of approximately 40 g is used, the retinol concentration C in baby porridge-powder containing milled cereals and fruit should be reported with the expanded measurement uncertainty, i.e. $C \pm 20\%$ of the measured value C (95% confidence).

When baby porridge-powder containing milled cereals and fruit is to be analysed, it is recommended to use a relatively large test sample of approximately 40 – 50 g and not 2 – 10 g as indicated in the official CEN method (EN-12823-1). As the analytical uncertainty (40 g test sample) was bigger than the normal analytical uncertainty of the laboratory, even a bigger test samples than 40 g might be considered.

9 Summary

Measurement Uncertainty –40 g test samples				Sample
	Sampling	Analytical	Total	Typical between target variation $RSD_B(\%)$ of the mean values of

				analyses of the batches in the validation test (see table 15)
Uncertainty u (%) = RSD (%)	4.95	8.3	9.7	8.2
[#] Expanded uncertainty U (%) = $2 \cdot u$	9.9	16.6	19.4	16.4

[#] With a coverage factor of 2 (i.e. 95% confidence)

Acknowledgement:

Nestlé (Norway) is thanked for their enthusiastic cooperation and in addition for providing samples to the project (validation and quality control study). Also Smaafolk - Tine Norske Meierier is thanked for kindly offering us samples to the quality control study. The National Institute of Nutrition and Seafood Research (NIFES) is thanked for the analytical contribution (analyses and information on the laboratory QA-system). The study is done with financial support from the Nordic Innovation Centre and the Norwegian Food Safety Authority.

10 ANOVA calculation, vitamin A in baby porridge - details

Calculation of uncertainty of analyses, one-way ANOVA, test sample 40 g

Table 14 - ANOVA calculations – uncertainty of analyses - sum of squares of differences, within groups (SS-Error)

Sample	Analyses (µg/100g)		Mean value – each sample, (µg/100g)	² Squares of differences – within groups (µg/100g) ²
	A1 = x _{1j} = x ₁₁	A2 = x _{1j} = x ₁₂	$\bar{x}_i = (x_{11} + x_{12})/2$	$(x_i - \bar{x}_i)^2$
B1-S1	402	325	363.5	1482.25
B2-S1	382	319	350.5	992.25
B3-S1	332	291	311.5	420.25
B4-S1	280	278	279	1
B5-S1	370	409	389.5	380.25
B6-S1	344	318	331	169
B7-S1	297	333	315	324
B8-S1	336	320	328	64
B9-S1	372	353	362.5	90.25
B10-S1	407	361	384	529
B1-S2	361	351	356	25
B2-S2	349	362	355.5	42.25
B3-S2	397	348	372.5	600.25
B4-S2	358	321	339.5	342.25
B5-S2	378	460	419	1681
B6-S2	381	392	386.5	30.25
B7-S2	341	315	328	169
B8-S2	292	306	299	49
B9-S2	332	337	334.5	6.25
B10-S2	322	382	352	900
Mean value of measurements			² SS-Error (SS _E):	
$\bar{X}_a = 1/20 * \sum_{i=1}^{20} \bar{x}_i = 347,85 \mu\text{g}/100 \text{ g}$			$= \sum_{i=1}^{20} [(x_{i1} - \bar{x}_i)^2 + (x_{i2} - \bar{x}_i)^2] = \sum_{i=1}^{20} 2 * (x_i - \bar{x}_i)^2$	
SS _E (µg/100g) ²	³ Degree of freedom (df) (N*2-N)=20	Variance = SS _E /df (µg/100g) ²	Standard deviation, SD _{anal} = $\sqrt{\text{SS}_E/\text{df}}$ (µg/100g)	Relative standard deviation RSD _{anal} (%) = (SD / \bar{X}_a)*100%
16595	20	829.75	28.80538	8.280978

² Calculation of SS-Error - in this case two test samples are analysed of each laboratory sample, therefore

$$(x_{i1} - \bar{x}_i)^2 = (x_{i2} - \bar{x}_i)^2 \Rightarrow \text{SS}_E = \sum_{i=1}^{20} [(x_{i1} - \bar{x}_i)^2 + (x_{i2} - \bar{x}_i)^2] = \sum_{i=1}^{20} 2 * (x_i - \bar{x}_i)^2$$

If the number of test samples analysed is n > 2, the squares of differences will be not be equal and the calculation

to be done is the following: $\text{SS}_E = \sum_{i=1}^{20} \sum_{j=1}^n (x_{ij} - \bar{x}_i)^2$

³ df = (N*n-N)=(20*2-20)= 20 there N is the number of samples and n is the number of test samples analysed of each batch

Calculation of uncertainty of sampling, one-way ANOVA, test sample 40 g

Table 15 - ANOVA calculations – uncertainty of sampling - sum of squares of differences

S1A1= x_{i1}	S1A2= x_{i2}	S2A1= x_{i3}	S2A2= x_{i4}	\bar{x}_i \bar{x}_i	$\left(\frac{x_{i1} + x_{i2}}{2} - \bar{x}_i\right)^2$	$\left(\frac{x_{i3} + x_{i4}}{2} - \bar{x}_i\right)^2$	
402	325	361	351	359.75	14.0625	14.0625	
382	319	349	362	353	6.25	6.25	
332	291	397	348	342	930.25	930.25	
280	278	358	321	309.25	915.0625	915.0625	
370	409	378	460	404.25	217.5625	217.5625	
344	318	381	392	358.75	770.0625	770.0625	
297	333	341	315	321.5	42.25	42.25	
336	320	292	306	313.5	210.25	210.25	
372	353	332	337	348.5	196	196	
407	361	322	382	368	256	256	
$4 \text{ } SS_{\text{Samp}} = \sum_{i=1}^{10} \left[\left(\frac{x_{i1} + x_{i2}}{2} - \bar{x}_i \right)^2 + \left(\frac{x_{i3} + x_{i4}}{2} - \bar{x}_i \right)^2 \right] = \sum_{i=1}^{10} \left[2 * \left(\frac{x_{i1} + x_{i2}}{2} - \bar{x}_i \right)^2 + 2 * \left(\frac{x_{i3} + x_{i4}}{2} - \bar{x}_i \right)^2 \right] = 14231$							
$SSE_{\text{Anal}} = 16595$ (see table 14)				$df_S = 10$ (see table note)			Mean value of all measurements $\bar{x} = 347.85$
Varians $V_{\text{Samp}} = (SSS/df_S - SSA/df_A)/2 = (14231/10 - 16595/20)/2 = 296.675$				$df_A = 20$ (see table 14)			
$SD_{\text{Samp}} = \sqrt{V_{\text{Samp}}} = 17.224$				$RSD_{\text{Samp}}(\%) = (SD_{\text{Samp}}/\bar{x}) * 100\% = 4.95\%$			

⁴ The difference d between the mean value \bar{x} of the two values $\left(\frac{x_{i1} + x_{i2}}{2}\right)$ and $\left(\frac{x_{i3} + x_{i4}}{2}\right)$ to each of the values are identical. The expression could therefore be written

$$\text{as } SS_{\text{Samp}} = \sum_{i=1}^{10} 4 * d_i^2 = \sum_{i=1}^{10} \left[4 * \left(\frac{x_{i1} + x_{i2}}{2} - \bar{x}_i \right)^2 \right]$$

⁵ $df_S = (N_B * n - N_B) = (10 * 2 - 10) = 10$ there N_B is the number of batches and n is the number of primary samples (= laboratory samples) analysed of each batch