Uncertainty of Measurement (UoM) applied to food analysis

ISO
9001
CERTIFIED

UNCERTAINTY OF MEASUREMENT (UoM) APPLIED TO FOOD ANALYSIS

SECTION 1 - INTRODUCTION

Background
This approach to providing a UoM assessment follows the requirements of BS EN ISO/IEC 17025:2005[1] and is based on the EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement[2] with reference also to the Guide to the Expression of Uncertainty in Measurement, (GUM)[3]. Uncertainty of measurement can be defined as "A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand.[3]

The food analysis example of the assessment of UoM described here is based on several years Quality Control (QC) data from the AOAC method for measuring Total Dietary Fibre (TDF)[4] in various food materials. Data and calculations are also given from other analytical methods to cover the options provided for in the approach adopted. The steps described below are based on in-house provision for uncertainty assessment for chemistry analytical methods at Campden BRI that are UKAS accredited, ensuring a valid, systematic and uniform approach to the assessment. This is one of a number of approaches that could be adopted, but it was judged by Campden BRI to be the most appropriate for chemistry analytical methods.

The essential steps for a valid UoM
Listed below are the obligatory steps that provide a valid UoM. To be in accord with the guidance in the EURACHEM/CITAC Guide[2] and the requirements of BS EN ISO/IEC 17025:2005, all of the steps noted below MUST be addressed in a document for audit purposes. In some cases the step may not be applicable, but the reasons for the step not being applied must be noted in the documentation for audit purposes. The documentation has two important functions. Firstly, it notes how the uncertainty in measurement results should be reported to a client. Secondly, the whole document provides an auditor with a complete record of the uncertainty assessment.

What follows is, firstly, a description and explanation for the steps in the process of uncertainty of measurement assessment with generalised examples based on the Campden BRI UoM in-house designed worksheet. A series of boxes are then provided to show the specific UoM results and requirements for the AOAC Total Dietary Fibre (TDF) analysis method. Appendices I and II give the format for documenting the uncertainty assessment for audit purposes and a listing of the statistical equations used in the calculations to provide the basis for an uncertainty of measurement value. The explanations for the calculations given in Section 3 have been cross referenced to the equations in Appendix II.

Below is a list of the steps that need to be addressed. Section 2 explains how each of these steps have been addressed in the Campden BRI approach to evaluating UoM:

1   **Measurand specification**
1.1   Scope
1.2   Method
1.3   Equation
2   **Identification of uncertainty sources**
3   **Quantification of uncertainty**
3.1   Simplification by grouping sources covered by existing data

SECTION 2 - EXPLANATION OF THE STEPS IN THE CAMPDEN BRI APPROACH TO UNCERTAINTY OF MEASUREMENT FOR CHEMISTRY ANALYTICAL METHODS

Step 1 - Specification of the measurand

The measurand must be specified. This requires:

1.1   An accurate description of what measurement the UoM applies to. The types of samples should be noted with the range of values that the UoM applies to. If applicable, any sources of uncertainty, recognised, but not included in the assessment, should be noted with an explanation as to why they have been excluded - (scope).

1.2   There must be a detailed account of the method, either referenced or recorded in full in the assessment. It may be found useful, in helping to define the sources of uncertainty, to record the main steps in the method here. This is not, however, obligatory - (method).

1.3   An equation to describe how the analytical quantity is derived - (equation).

Box 1 in Section 4 contains the required information for step 1 of the UoM assessment for the total dietary fibre example.

Step 2 - Identification of the sources of uncertainty

This requires a description of the sources of uncertainty in the method. A cause and effect ('fishbone' or Ishikawa) diagram is generally recommended for identifying the sources (see reference 2, p100). In the approach described in this report, however, a flow diagram has been used as the primary basis for assessing sources of uncertainty with the 'cause and effect' diagram attached to the particular analytical method step. Sequential tabulation of the elements of the diagram can be used, if the flow diagram becomes too complicated. This approach has been taken because chemistry methods are nearly always sequential, and this way of expressing the sources of uncertainty leads to less likelihood of missing parts of the method, compared with the fishbone approach. **Figure 1** gives a generalised picture of the way in which the diagram is constructed.

Figure 1   Generalised flow diagram for identifying sources of uncertainty
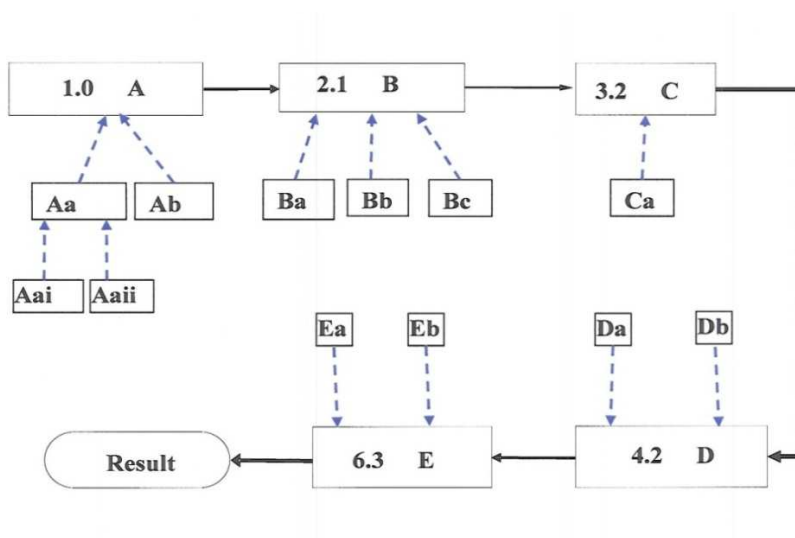
Uncertainty in the final **'Result'** arises from uncertainty in flow diagram stages A, B, C, D and E. A is itself uncertain because of Aa and Ab; B is uncertain because of Ba, B. Uncertainty factors Aa etc. can themselves have contributory uncertainty components (e.g. Aai, Aaii,…) and in principle this could go on forever.

The numbers in the top level boxes e.g. **'1.0'** are references to the numbered sections in the description of the analytical method (e.g. Standard Operating Procedure). This referencing makes it easy to trace back uncertainty sources to specific points in the method.

 The recursion is stopped when it is considered that:

a)   components being added make negligible contribution compared to those already added (say less than one-tenth of the largest), or

b)   the 'compound' uncertainty can be estimated more easily than the components. For example, if the uncertainty in 'A' can be estimated more easily than that in Aa then Aai and Aaii do not need to be considered; Aa and Ab should still be listed to ensure and show that any substantial contributors to the uncertainty in A have not been omitted. It is thus essential, to be in accord with BS EN ISO/IEC 17025:2005, that there is a complete list of uncertainties even if the compounding of the sources of uncertainty is adopted, using QC data (see 3.1 within step 3). When identifying sources of uncertainty for chemistry analytical methods, the experience at Campden BRI has been that the third level of uncertainty (e.g. Aai, Aaii) is seldom required.

The Eurachem guide[2] lists (noted below) the following typical sources of uncertainty which can be used for the cause and effect parts of the diagram, associated with the flow diagram stages.

- Sampling
- Storage conditions
- Instrument effects
- Reagent purity
- Assumed stoichiometry
- Measurement conditions
- Sample effects
- Computation effects
- Blank correction
- Operator effects
- Random effects

Such a check list is useful when reviewing or compiling a list of uncertainty sources, to ensure that none have been missed.

Records should clearly identify what is meant by the source (e.g. "water loss by evaporation during weighing") and - if it is not obvious - some qualitative indication of its effect on the result (e.g. "increase in measured fat content due to reduced water content").

Where analytical methods are similar the relevant uncertainty sources may also be similar. In that case individual method assessments may document uncertainty sources by reference to a common list. However, during the uncertainty assessment of each method it is still essential to review the uncertainty sources to ensure that the common list is appropriate and complete, and to record that review for audit purposes.

Box 2 in Section 4 shows the sources of uncertainty, expressed as flow diagram stages with associated cause and effect diagrams, for the AOAC Total Dietary Fibre (TDF) method.

Step 3 - Quantification of uncertainty

3.1   Simplification by grouping uncertainty sources covered by existing data

The aim of the process described below is to evaluate the random and systematic effects on measurement results, by obtaining estimates of precision and bias for the whole analysis, rather than attempting to evaluate individual uncertainty components. There may be relevant internal or external quality control or validation data already available for established methods. This may include, for example, data from replicate analysis of samples, analysis of reference materials, participation in proficiency trials or ring trials. Information on common 'unit operations' (e.g. weighings, or making up of standard solutions) may be available from QC data, or from other uncertainty evaluations .

a)   To the extent that such data sets include representative (neither exaggerated nor minimised) variation due to identified uncertainty sources, they may be used to assess the (grouped) uncertainty due to those sources. The aim of the uncertainty evaluation is, as far as possible, to reflect the likely uncertainty in results obtained when the method is being operated correctly and is under statistical control. Failure to follow the method as specified should not be included. Such events should be prevented or detected by other parts of the quality system.

Such data, however, may <u>exclude</u> some uncertainty sources. For example:

- Sample replication may be accomplished by division of the first suspension/solution of the sample, thus excluding variation due to sampling of the analytical unit and preparation of that first suspension/solution.

- Sample replicates may be analysed as part of the same batch by the same operator on the same day, thus excluding variation due to operator, day and factors such as batches of chemicals, calibrations etc.

- Reference materials or materials from proficiency testing schemes may have a matrix that is different from the food samples being analysed, thus excluding variation and bias due to the effects of the sample matrix and/or sub-sampling.

- It is possible to provide an uncertainty estimate with precision data only (repeat analyses of samples) but ideally, bias data (from analysis of a reference standard with a 'true' value) should be included. Information on bias should be provided if it is available. If bias has not been assessed then this must be clearly stated in the uncertainty report.

It is most important to recognise, and to document any components of uncertainty that are not covered by the existing data. Uncertainty sources whose effects are not appropriately included in the data must be evaluated separately (see 3.3 of step 3) or excluded from the uncertainty estimation (any such exclusion must be made clear in any uncertainty statement).

Over time it may be possible to obtain data for any components not initially accounted for through the analysis of additional quality control samples.

b) For methods under development, or other methods where there is inadequate existing data, it may be appropriate to plan experiments to produce suitable data. In that case, the considerations in (a) above should be taken into account.

> **Box 3** in Section 4 describes the QC data provided to quantify the grouped sources of uncertainty for the total dietary fibre method.

3.2 Quantification of grouped components

At Campden BRI a calculation sheet is provided within the in-house EXCEL workbook, and in **Figures 2 to 5** the various elements of the calculation sheet are reproduced and described. **Boxes 1 to 5** summarise the UoM procedure for the AOAC Total Dietary Fibre analysis method.

3.3 Quantification of remaining components

If uncertainty components are identified which are not adequately covered by the available data obtained in 3.1 of step 3 an attempt should be made to evaluate them separately (see reference 2, Section 7.10). Any components not covered by available data, or evaluated separately must be clearly mentioned in the documentation of the uncertainty estimation.

3.4 Conversion of components to standard deviations

Calculations are provided within the worksheet to give the required standard deviations (The text in Section 3 has been cross-referenced with the equations noted in Appendix II).

Step 4 - Calculation of the combined uncertainty

Again, the calculations required to achieve this step are contained within the in-house worksheet. If the appropriate data have been provided (see **Box 3** in Section 4) then the worksheet will generate two plots, one for precision and one for bias (see **Figures 3 and 4**). From the shape of the distribution of the data in these plots, a decision is made as to whether the uncertainty should be expressed as a **Relative** or **Absolute** value (see **Figures 3** and **4** and **Box 4**).

4.1 Calculation of the combined standard uncertainty

The worksheet then provides the combined uncertainty, obtained from the precision and bias data. This will either be a **Combined Relative Uncertainty** or a **Combined Absolute Uncertainty**, depending on the decision made with respect to the data patterns in the precision and bias plots. In addition, a calculation is made, based on a threshold, that delivers a comment as to whether the bias and its uncertainty are negligible compared to the estimate of precision. The various options used by Campden BRI for the **'non-negligible'** or **'negligible'** bias conditions are described and explained in **Figures 5(a)** and **5(b),** and **Box 5**.

4.2 Calculation of the expanded uncertainty

This calculation is the last step in defining the uncertainty value that will be applied to measurement results. (see **Figures 5(a)** and **5(b)).** It provides an uncertainty value which will be valid for the stated confidence level (typically a confidence level of 95% is used).

Step 5 - Recording and reporting uncertainty

To satisfy an auditor it is not enough just to record the result of the uncertainty calculation. All the essential steps for a valid UoM described above must be documented as noted in Appendix I, for the benefit of an auditor. The way in which the uncertainty value is reported, say to a client, takes a specific form, and the approach used by Campden BRI is also given in Appendix I.

Step 6 - Review

To meet accreditation requirements, laboratories should have procedures in place for documenting and reviewing uncertainty estimates and for the storage and retrieval of data.

6.1 Date of this assessment

The date of the assessment should be recorded and the name of the person doing the assessment.

6.2 Date for the review of this estimation

A date for review of the uncertainty for the method should be noted in the documentation. Campden BRI has specified a period of three years from the last assessment. If the method is changed in a way that could affect uncertainty, then the measurement uncertainty should be reviewed before the set period.

Step 7- File storage location

The calculation and associated worksheets (raw data, sources of uncertainty, etc), and the documented assessment must be archived permanently and the location must be stated in the documentation.

SECTION 3 - EXPLANATION OF THE OPERATIONS IN THE CALCULATION WORKSHEET.

Brief explanations are given below for each of the calculations in the in-house worksheet required to calculate uncertainty. The calculations worksheet has been constructed to generate, immediately, appropriate results, including the precision and bias plots, when the data are entered into the appropriate fields in the worksheet (see **Box 3** in Section 4 for an example of the data entry worksheet).

Figures 2(a) and 2(b) show typical outputs from the worksheet. The terms that appear in the worksheet are described below.

Figure 2(a)   **Terms used in the calculation of uncertainty of measurement (relative uncertainty) - data from LCMS tricothecene analyses**

Precision s.d.(Absolute)	15.5284	**Combined Relative Uncertainty**				
Precision (Relative)	**0.1884**	Standard Uncertainty	0.1906			
Degrees of Freedom	118	Confidence	95%			
Bias (Absolute)	-7.0393	Degrees of Freedom	123.4494			
s.e. Bias (Absolute)	1.9246	Coverage Factor (k)	1.9794			
Bias (Relative)	**-0.0425**	Expanded Uncertainty	0.3773			
s.e. Bias (Relative)	**0.0286**	Bias is NOT negligible	**CONSULT YOUR LINE MANAGER!**			
Degrees of Freedom	126	**Include** bias in uncertainty calculation				
9 distinct samples	127 results	Calculate **Relative** uncertainties				

Figure 2(b)   **Terms used in the calculation of uncertainty of measurement (absolute uncertainty) - data from Weibull-Stoldt fat analyses**

Precision s.d.(Absolute)	**0.1272**	Combined Absolute Uncertainty			
Precision (Relative)	0.0606	Standard Uncertainty	0.1272		
Degrees of Freedom	45	Confidence	95%		
Bias (Absolute)	**0.167**	Degrees of Freedom	45	Absolute Bias	0.167
s.e. Bias (Absolute)	**0.0325**	Coverage Factor (k)	2.0141	Expanded Uncertainty	0.0682
Bias (Relative)	0.0153	Expanded Uncertainty	0.2563		
s.e. Bias (Relative)	0.0029	Bias is NOT negligible	**CONSULT YOUR LINE MANAGER!**		
Degrees of Freedom	19	**Ignore** bias in uncertainty calculation			
27 distinct samples	72 results	Calculate **Absolute** uncertainties			

Explanation of terms used in the calculation of uncertainty of measurement

The equations given in Appendix II have been cross-referenced in brackets after the section titles below.

Precision (1.0)

Precision data are generated from multiple analyses of a number of samples. Samples from customers, QC materials, or reference materials can be used to provide precision data. Ideally samples, representative of the range of samples defined by the method scope, should have been

analysed. Precision is essentially a measure of how closely results from the replicate analyses of a particular sample are grouped. A high degree of precision does not necessarily mean a high degree of accuracy, as results may be precise but biased. The precision value presented in the worksheet is a 'pooled' value based on a combination of the precision estimates for individual samples. The **Relative Precision** value is often multiplied by 100 to express as a percentage. The **Relative Precision** is not in the same units as Results. It must be multiplied by a Result (or the average result for a sample) to give precision in the same units as a Result.

Precision s.d. (relative/absolute) (1.3/1.2)
The in-house worksheet presents the uncertainty due to measurement precision in, two forms: **Precision s.d. (Absolute)** and **Precision (Relative).** Based on the distribution of the data in the precision and bias plots (see **Figures 3** and **4** and **Box 4**) the analyst selects whether a relative or an absolute uncertainty is most appropriate. Based on this selection, the appropriate values become emboldened in the worksheet to indicate which values are being used in the calculations.

If the precision is approximately proportional to the analyte level (i.e. the magnitudes of deviations of results from sample means are approximately proportional to the sample means) then the '**Precision (Relative)**' is used. This will result in a '**Combined Relative Uncertainty**' (see **Figure 2(a))**.

If the precision is approximately constant, regardless of the analyte concentration (i.e. if the magnitudes of deviations of results from sample means are about the same for different sample means), then the '**Precision s.d. (Absolute)** is the most appropriate option, giving a **'Combined Absolute Uncertainty'** (see **Figures 2(b) and 3**).

The Relative/Absolute choice cell
The formatting and commands within the worksheet were constructed to produce several responses.

a)   When the decision has been made to describe the data distribution as **'Absolute'** or **'Relative'** then the cell seen in the worksheet extract (see **Figure 2(a)** and **2(b)**) can be 'toggled' for either of these conditions.

b)   If **'Absolute'** is chosen then the title at the top of the column will read **'Combined Absolute Uncertainty'** (see **Figure 2(b))**.

c)   If **'Relative'** is chosen then the title at the top of the column will read **'Combined Relative Uncertainty'** (see **Figure 2(a)**).

d)   To further clarify which statistical data are being used, the appropriate statistical values are emboldened (see **Figures 2 (a)** and **2 (b)**), since both sets of statistics are provided.

Figure 3   The precision plot - absolute uncertainty - analysis of starch data
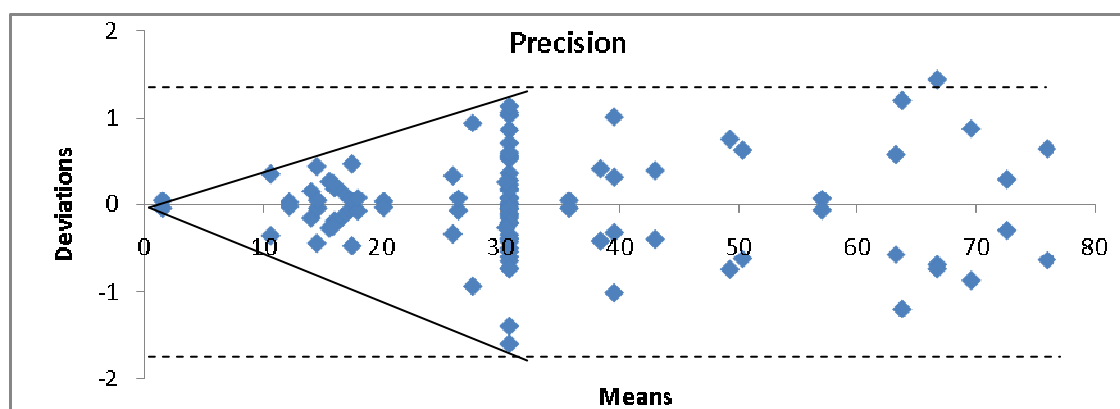
The precision and bias plots produced by the worksheet are used by the analyst to determine which form of uncertainty - absolute or relative - is most appropriate. An absolute uncertainty is a single fixed figure, used where the data pattern indicates that the variation in results is similar across the range of analyte concentrations. In the starch analysis example shown in Figure 3, however, the data range could have been split into two, from 0 - 30, showing a **relative uncertainty** pattern (solid plot lines) and from 30 - 80, showing an **absolute uncertainty** pattern (dotted plot lines). The decision was made, however, to use an absolute uncertainty for the whole range. This leads to a less ambiguous UoM at the expense of an exaggeration of the uncertainty for the smaller values. See **Box 4** in Section 4 for an illustration of data leading to the use of relative uncertainty.

Figure 4   The bias plot - absolute uncertainty - analysis of starch data

The bias plot in Figure 4 shows data obtained from the repeated analyses of two reference standards for starch. The plot indicates that an absolute uncertainty is the most appropriate form in this case. The bias plot also shows that it would be appropriate to increase the number of reference standard values over the range of results.
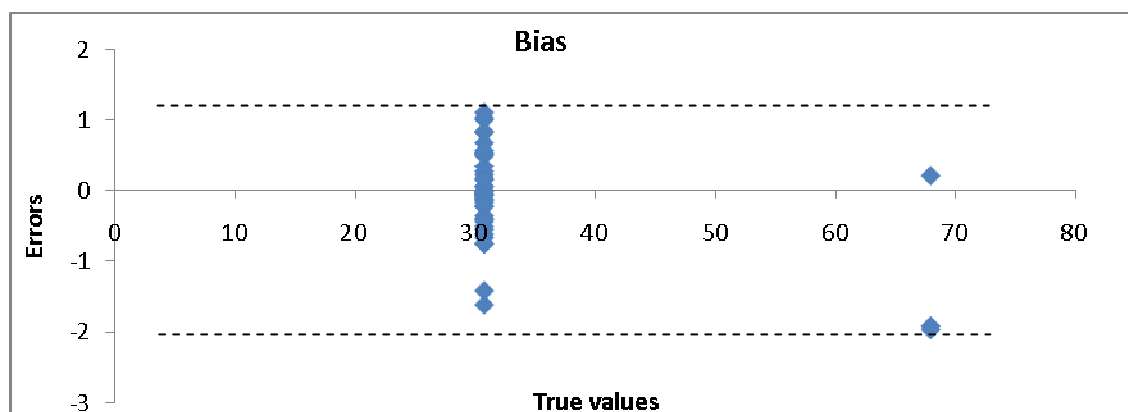
Degrees of freedom (Precision) (1.1)
Degrees of freedom for the estimate of the precision standard deviation are related to the number of results and samples used to calculate the particular statistic.

Bias (Relative/Absolute) (2.4/2.2)
Bias is estimated by comparing measurement results with a 'true value'. The data will be derived from analysis of Certified Reference Materials (CRMs), in-house prepared reference materials, or 'spiked' samples.

'**Bias (Absolute)'** is calculated as the average of (result minus "true" value). If the bias plot indicates that the magnitudes of the errors of results from the "true" values are about the same for different "true" values, then the absolute option should be adopted (see **Figures 2(b) and 4**).

'**Bias (Relative)**' is estimated as the average of [(result minus "true" value)/"true" value]. This is not in the same units as the measurement results. It must be multiplied by a result (or a "true" value) to give bias in the same units as a measurement result. This is the appropriate measure of bias if the magnitudes of error between results and "true" values are approximately proportional to sample means (see Box 4 in Section 4). This statistic will be employed when the **'Relative'** option is used (see **Figure 2(a)**).

s.e. Bias (Relative/Absolute) (2.5/2.3)
The standard error on the estimate of the bias is displayed as both '**s.e. Bias (Absolute)**' and '**s.e. Bias (Relative)**'. One of these values will appear emboldened in the worksheet, depending on the option adopted in the cell that can be 'toggled' for the two conditions (absolute/relative). (See figures 2(a)and 2(b))

Degrees of Freedom (Bias) (2.1)
The degrees of freedom associated with the estimate of bias are related to the number of results used for the generation of the particular statistic.

Distinct samples results
Samples are recognised as distinct when they have different sample names. The number of results is a summation of replicate analyses of samples (to estimate precision) and the number of analytical results with their 'true' values (to estimate bias).

Combined absolute/ relative uncertainty
(Combined uncertainty)

When the in-house worksheet contains no data, the central data column is titled **'Combined Uncertainty'**. When data are entered and **'Absolute'** or **'Relative'** uncertainty is selected, then the column becomes titled **'Combined Absolute Uncertainty'** or **'Combined Relative Uncertainty'**.

In the starch example (**Figures 3** and **4**) '**Absolute Uncertainty'** has been selected based on the precision and bias plots. The range could have been split to provide two uncertainty values, but the decision was made to provide only one UoM value for the whole range.

In the TDF example section (see **Box 4**) the '**Relative Uncertainty'** has been selected, based on the precision and bias plots. The worksheet therefore displays the **'Combined Relative Uncertainty'** (see Box 5).

Standard combined uncertainty (3.1) (3.5)

The value calculated for the '**Standard Uncertainty**' (3.1), will depend on whether the uncertainty associated with the bias has been included in the uncertainty calculation. This decision is made based on whether the bias and its uncertainty is considered negligible (3.5) compared to the estimate of precision (see **Ignore/include bias choices** below). The standard uncertainty will be expressed as an absolute or a relative value (3.1) depending on the option adopted in the cell that can be 'toggled' for the two conditions (absolute/relative).

Confidence
The confidence level influences the coverage factor (k) which is used to calculate the expanded uncertainty. The confidence level may be reduced or increased but for most applications the confidence level is 95%, as in the examples shown in this report.

Degrees of freedom (Combined Uncertainty) (3.2)
The degrees of freedom associated with the combined uncertainty relate to the degrees of freedom for the precision, and the bias (if included) estimates. The value for degrees of freedom for the combined uncertainty is dependent upon whether the absolute or the relative condition has been selected.

Coverage factor (k) (3.3)
The coverage factor used to calculate the expanded uncertainty is obtained from the Student t distribution and depends on the confidence level chosen and the degrees of freedom. For a confidence level of 95% the coverage factor, k. is typically in the range 1.96 to 3.

Expanded uncertainty (3.4)
The combined standard uncertainty is multiplied by the coverage factor to obtain an expanded uncertainty. This is a quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand. The expanded uncertainty is reported for a specified confidence level and is obtained by multiplying the combined uncertainty (absolute or relative) by the coverage factor.

Ignore/include bias choices (3.5, 3.7)
A threshold has been set so that if the estimated bias and its uncertainty exceed 1/5th of the precision s.d, then the bias is defined as **'NOT negligible' (see Figures 5(a)** and **5 (b))**. This can be regarded as a stringent threshold, and a threshold of 1/3rd would be acceptable. A cell is also provided which gives the analyst the choice to '**Ignore**' the bias or '**Include**' the uncertainty associated with the bias (see **Figures 5(a)** and **5 (b))**.

If the '**Include**' option is chosen, the bias uncertainty (i.e. s.e bias) is included in the combined uncertainty and the right-hand extended box remains blank (see **Figure 5 (a)**). If this option is chosen, when reporting the result for a test sample and its uncertainty, the result must be corrected for the non-negligible bias.

If the **'Ignore'** option is adopted the bias uncertainty is not included in the combined uncertainty estimate. Instead, the values for the bias and the expanded uncertainty are shown opposite the cell titles noted (see **Figure 5(b)** and equation **(3.7)**). These values must be entered in the assessment document with the **'Expanded Uncertainty'** in the column to the left in **Figures 5(a)** and **5(b)**. **'Absolute Bias'** or **'Relative Bias'** and **'Expanded uncertainty'** labels are provided on the extended box in the worksheet extract (see **Figure 5 (b)**) The labels **'Absolute bias'** or **'Relative bias'** appear, according to the choice based on the precision and bias plots.

If the threshold is not exceeded then the cell returns **'Bias is negligible'**. When the '**Ignore**' bias option is selected, it is not included in the uncertainty estimate. If the '**Include**' option is used, then the negligible bias is included in the final uncertainty value. It is, however, quite in order to neglect a negligible bias completely.

Figure 5 (a)   Uncertainty calculations for fat analysis by Weibull-Stoldt to illustrate non-negligible bias and the option to include the bias

Precision s.d.(Absolute)	**0.1272**	**Combined Absolute Uncertainty**		
Precision (Relative)	0.0606	**Standard Uncertainty**	0.1314	
Degrees of Freedom	45	**Confidence**	95%	
Bias (Absolute)	**0.167**	**Degrees of Freedom**	50.5777	
s.e. Bias (Absolute)	**0.0325**	**Coverage Factor (k)**	2.0085	
Bias (Relative)	0.0153	**Expanded Uncertainty**	0.2639	
s.e. Bias (Relative)	0.0029	Bias is NOT negligible	**CONSULT YOUR LINE MANAGER!**	
Degrees of Freedom	19	**Include**	bias in uncertainty calculation	
27 distinct samples	**72 results**	Calculate	**Absolute**	uncertainties

When the bias uncertainty is calculated as **'not negligible'**, advice is given to **'Consult your line manager'**. The analyst, in consultation with their manager, has the option to '**Include**' or '**Ignore**' the bias. In the example shown in Figure 5(a) the bias uncertainty has been included with the combined absolute uncertainty (standard uncertainty), and thus the extended cells to the right remain unfilled. If the option is chosen to **'Include'** the bias then, when reporting the result for a test sample and its uncertainty, the result must be corrected for the non-negligible bias by subtraction of the bias figure from the result[a]. This is the procedure for allowing for bias in the case of 'absolute uncertainty'. When the 'relative uncertainty' condition is used, then a different rule applies[b].

---

[a] In the case of 'absolute uncertainty': When the Bias sign is +ve the value is subtracted from the result. If the value is -ve then the value is subtracted from the result.

[b] In the case of 'relative uncertainty': The result must be divided by 1 + Bias, if the Bias (Relative) has a positive sign, 1-Bias, if the Bias (Relative) has a negative sign

Figure 5 (b)   Uncertainty calculations for fat analysis by Weibull-Stoldt to illustrate non-negligible bias and the option to ignore the bias

Precision s.d.(Absolute)	**0.1272**	Combined Absolute Uncertainty			
Precision (Relative)	0.0606	Standard Uncertainty	0.1272		
Degrees of Freedom	45	Confidence	95%		
Bias (Absolute)	**0.167**	Degrees of Freedom	45	**Absolute Bias**	0.167
s.e. Bias (Absolute)	**0.0325**	Coverage Factor (k)	2.0141	**Expanded Uncertainty**	0.0682
Bias (Relative)	0.0153	Expanded Uncertainty	0.2563		
s.e. Bias (Relative)	0.0029	Bias is NOT negligible	**CONSULT YOUR LINE MANAGER!**		
Degrees of Freedom	19	**Ignore**	bias in uncertainty calculation		
27 distinct samples	**72 results**	Calculate	**Absolute**	Uncertainties	

Advice may be given to ignore the bias for practical reasons. It is important, however, to document the reason(s) for this decision. If the option to **'Ignore'** bias in the uncertainty calculation' is selected in the cell that can be 'toggled' for either condition, then the **'Absolute Bias'** and **'Expanded Uncertainty'** labels appear in the extended cells on the right, and values are calculated (i.e. the bias and the associated expanded uncertainty for the bias estimate are recorded separately from the combined uncertainty). The 'Absolute Bias' and its 'Expanded Uncertainty' must be documented in addition to the 'Expanded Uncertainty' in the central column (which is only based on precision data). When results are reported to customers the bias and the expanded uncertainty for the bias must be given.

If the 'Ignore' and 'Include' options are used when the Uncertainty is Relative, then all references to 'Absolute' are replaced with 'Relative', and different values are returned to some of the cells.

SECTION 4 - A SPECIFIC UoM FOOD ANALYSIS EXAMPLE - THE ANALYSIS OF TOTAL DIETARY FIBRE BY THE AOAC METHOD

The following series of boxes show the specific information and data that is required for the AOAC Total Dietary Fibre assessment of Uncertainty.

Box 1 Specification of the measurand

1.1 Scope

The AOAC procedure for analysing Total Dietary Fibre (TDF) (TES-AC-203)[*]. The results apply to the sub-samples taken from the sample supplied by the client. The method is applicable to general foodstuffs.

1.2 Method

Two sub-samples (dried if necessary, defatted if containing over 10% fat, and de-sugared if containing over 50% sugar) are enzymatically digested to remove protein and starch. After precipitation of soluble dietary fibre, the total residue is dried and weighed. One sub-sample is analysed for protein and the other for ash. Total Dietary Fibre (TDF) is calculated as the weight of residue minus the weight of protein plus ash in the residue.

1.3 Equation

$$TDF\ (\%) = \frac{(Rmw\ -\ Pw\ -\ Aw\ -\ B)}{Sw(mean)}\ x\ 100$$

Where:
Rmw = Mean weight in mg of two dried subsample residues
Pw = Weight in mg of protein
Aw = Weight in mg of Ash
B = Residue, Protein, and Ash weights for blank sample calculated as Blank fibre value, mg B = Rmwb – Pwb – Awb
Sw = mean weight of two sub-samples

---

[*] This is a unique Campden BRI code for this method of analysis, and a similar unique name should be included in the scope or the full method should be included in the documentation.

In **Box 2** below, two additional methods (protein and ash analysis) are included in the analysis of total dietary fibre. These require a separate assessment of sources of uncertainty and those sources have been noted in the last stages of **Box 2**.

Box 2   Sources of Uncertainty for the analysis of TDF

```
Drying may or may not be required
```

| 6. Storage of Customer bulk | → | 6. Mix and homogenise two sub-samples | → | 6.2 Dry oven dish | → | 6.3 Cool and Weigh dish | → | 6.4 Weigh sub-samples |

a) Temperature control
b) Moisture loss

a)   Homogeneity

a) Balance precision
b) Balance calibration error

a) Dessicant not dry
b) Balance precision
c) Balance calibration error

a) Balance precision
b) Balance calibration error

```
De-fatting and or de-sugaring may or may not be required
```

```
Steam bath only required when sample is very wet
```

| 7.3 De-sugaring of sub-samples | ← | 7.1 - 7.3 De-fatting of sub-samples | ← | 6.7 Weigh dried sub-samples | ← | 6.6 Oven dry sub-samples and cool | ← | 6.5 Steam bath partial drying |

a) Balance precision
b) Balance calibration error
c) Loss of solid

a) Loss of solid

a) Balance precision
b) Balance calibration error

a) Oven temperature
b) Drying time
c) Efficiency of desiccator

a) Loss of sample from bubbling

| 7.4 - 7.18 Addition of starch and protein enzymes to sub-samples | → | 7.11 Precipitation of proteins with IMS from subsamples | → | 7.14 - 7.15 Filtration of the residues | → | 7.17 - 7.18 Dry and weigh the residues |

a) Too little incubation
b) Enzyme strength and amount

a) Incomplete precipitation

a) loss of residue
b) Risk of contamination

a) Oven temperature b) Drying time
c) Efficiency of desiccator
d) Balance precision
e) Balance calibration error

| **7.20 (TES-AC-087 - protein analysis)** | ← | Sub-sample 1 |

See UoM documentation for TES-AC-087

(Result % TDF)

| **7.19 (TES-AC-086 - Determination of Total Ash)** | ← | Sub-sample 2 |

See UoM documentation for TES-AC-086

Box 3   QC data for Total Dietary Fibre (TDF) analysis enabling the grouping of sources of uncertainty from Box 2

Reference	Analysis	'True value'
A	3.82	3.83
A	3.6	3.83
A	3.37	3.83
A	3.6	3.83
A	3.37	3.83
A	3.63	3.83
A	3.46	3.83

For each batch of analyses performed an appropriate reference material (see below) is analysed with the samples. The data set used to estimate the uncertainty for TDF determinations was obtained from the analysis of reference materials over two years. A small sub-set of typical data is provided opposite.

Sample code	Analysis	'True value'
D2	13.4	
D2	13.9	
D3	2.6	
D3	2.6	
D4	2.2	
D4	2	
D5	4.49	
D5	4.65	
D6	2.47	
D6	2.23	

In addition client samples have been analysed in duplicate for QC purposes. Examples of this data are shown opposite.
The QC data provided is considered to cover all sources of uncertainty. If any source of uncertainty was not accounted for, then it should be evaluated separately or its absence must be made clear in the documentation (see Appendix I).

To assess bias, reference materials with 'true' values are required. When using reference material data, the 'true' values should be reasonably evenly spread over the range of values expected for tests samples, with replicate results for each of the 'true' values.

In order to provide appropriate data for the uncertainty of measurement calculation it is recommended that some test samples are stored, under appropriate conditions, and that with each analytical batch one or two of these samples are re-analysed, giving replicate analyses over time, thus enhancing the precision data.

Three analysts produced the data for the determination of fibre using the AOAC method. If only one analyst had worked on the method, then this must be stated in the report as a source of variation not accounted for. The method includes alternative treatments according to food types, e.g. de-fatting, de-sugaring, steam bath drying. All of these treatments have been applied to the wide range of samples in the data set. If any had not been used, it would be important to note this in the documentation.

The data cells shown above are provided in the in-house resource calculation sheet. A raw data sheet is provided in the workbook before the calculation sheet, since the data may well have to be re-ordered or sample names re-coded, and these operations are best carried out in a simple worksheet rather than the calculation sheet. It is also important to identify the source of the data unequivocally for audit purposes, and this is best done in the raw data sheet.

In order to estimate bias and its standard error at least one sample must have a "true" value.
The choice of a true value for a sample should be clear. For example, the assigned value of a proficiency test sample. If not, it is likely that the sample is not suitable for bias estimation. QC data should encompass the provision of suitable data for bias and precision uncertainty estimation, although it is possible to obtain an Uncertainty of Measurement with Precision data alone, bias data must be provided whenever possible. If it is absent this must be made clear in the report.

Details of the types of materials used to generate the data for the evaluation of uncertainty for total dietary fibre analysis

Four reference standards were used over the two years the data was acquired. Each standard was used until the stock became exhausted. A new reference material was then acquired.

1.   An in-house prepared oat grain reference standard (the standard material is repeatedly analysed and a range of values established for the material). The median value is accepted as the 'true' value. The 'true' value was established as 9.55% Total Dietary Fibre (TDF).

2.   An in-house prepared cereal standard, prepared as noted in 1. The 'true' value was established as 9.92% TDF.

3.   A FAPAS extruded cereal product. The 'true' value is established from a number of laboratory test results. Again the median of the range is used. The 'true' value was 5.14 % TDF

4.   A FAPAS wheat flour, the data provided as in 3. above. The 'true' value was 3.83 % TDF.

The in-house materials provide a check that the bias has not changed significantly since the materials were characterised. The FAPAS materials, however, provide an independent bias check as they have independently assigned values. Results from the reference standards are used to evaluate both precision and bias and the duplicate customer samples give additional precision data.

The duplicate analyses were derived from customer samples consisting of a wide range of food materials including:

> - Cakes
> - Confectionary
> - Biscuits
> - Cereal products
> - Desserts
> - Meat products
> - Canned goods
> - Fish
> -Cheese

**Box 4 Precision and Bias plots in the calculations worksheet for the AOAC TDF example method -
Relative Uncertainty**

The precision data is derived from analysing in duplicate one customer sample in ten, or one per batch if less than ten, as well as duplicate analyses of the in-house standard with each batch of client samples. The precision and bias plots determine which form of uncertainty (absolute or relative) is most appropriate. If the distribution of the data gives the form of a 'trumpet', indicating that the precision is approximately proportional to analyte concentration, then a relative uncertainty will be most appropriate (i.e. the value of the uncertainty will increase as the value of the analytical result increases). In the case of the TDF data, both the precision and the bias plots (see below) show the 'trumpet' distribution, indicated by dotted lines. The plots indicate that it is appropriate to express the uncertainty as a relative value (note that relative uncertainties are often expressed as a percentage).
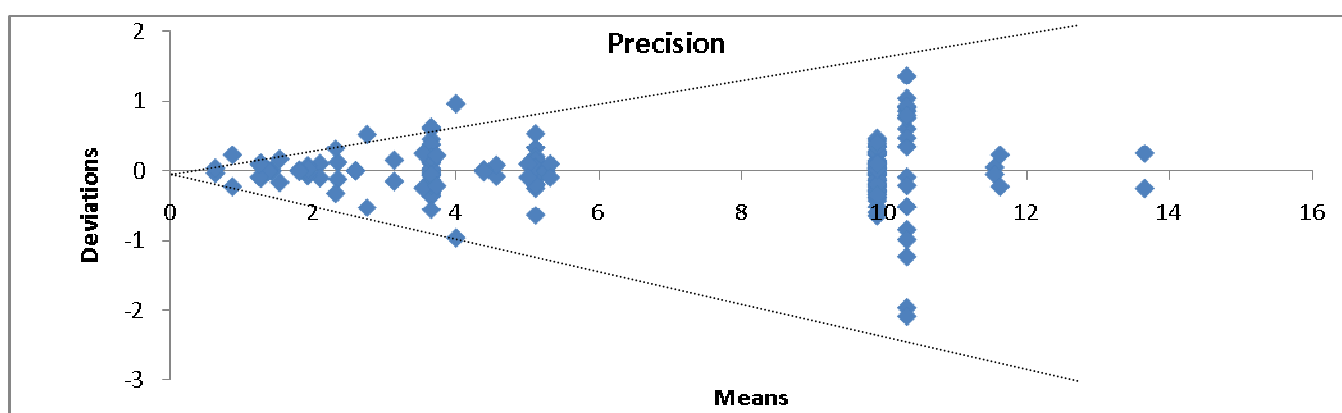
The bias data is derived from repeated analysis, over time, of in-house prepared reference materials and FAPAS reference material using the AOAC total dietary fibre analyses. The 'true' values for the reference standards entered into the worksheet (see data entries in **Box 3**) are used to calculate the bias.
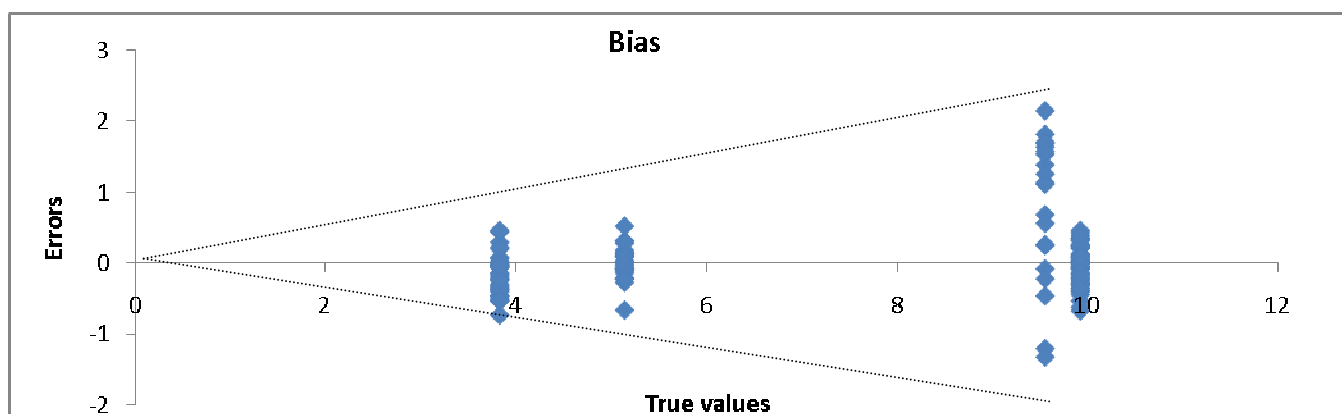
Box 5 Relative uncertainty with negligible bias, using the data from AOAC TDF method

Precision s.d.(Absolute)	0.4423	**Combined Relative Uncertainty**			
Precision (Relative)	**0.0764**	**Standard Uncertainty**	0.0764		
Degrees of Freedom	159	**Confidence**	95%		
Bias (Absolute)	0.0526	**Degrees of Freedom**	159		
s.e. Bias (Absolute)	0.0444	**Coverage Factor (k)**	1.9749		
Bias (Relative)	**-0.0019**	**Expanded Uncertainty**	0.1508		
s.e. Bias (Relative)	**0.0059**	Bias is negligible			
Degrees of Freedom	143	**Ignore** bias in uncertainty calculation			
31 distinct samples	**190 results**	Calculate **Relative** uncertainties			

In this example, the threshold (1/5th) for bias and its uncertainty compared with the precision s.d, has not been exceeded so the bias is identified in the worksheet as 'negligible'. The decision has been made to use a relative uncertainty, based on the precision and bias plots (see Box 4). The 'Include' or 'Ignore' toggled cell is primarily applicable to non-negligible bias. The 'Include'/Ignore' options do, however, work for a negligible bias and the figures will change accordingly. The separate bias figures are not, however, calculated or displayed in the right-hand box as they would be with non-negligible bias.

The final value for the expanded uncertainty, after multiplication by the coverage factor, to give a confidence level of approximately 95% is 0.151. The expanded uncertainty, expressed as a percentage, is 15.1%.

The expanded uncertainty for the result for a particular sample, expressed as an absolute value is calculated as follows (note that the Total Dietary Fibre results are expressed as %(w/w)):

> Sample D, result = 11.61 %w/w fibre
> Expanded uncertainty = 11.61 x 0.151 = 1.753 %w/w

.

References

1. BS EN ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories, British Standards Institution, 2005, ISBN 0580463303

2. EURACHEM/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, QUAM:2000.1, Second Edition, 2000. ISBN 0 948926 15 5

3. BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML. Guide to the Expression of Uncertainty in Measurement. International Organisation for Standardization Geneva, Switzerland. ISBN 92-67-10188-9, First Edition 1993

4. Lee SC, Prosky L, DeVries J. Determination of soluble and insoluble dietary fiber in foods. Enzymatic gravimetric method, MES-TRIS buffer: collaborative study. Method 991.43 J AOAC Int;75:395-416. 1992

Acknowledgement

APPENDIX I   DOCUMENTING THE UNCERTAINTY ASSESSMENT

UNCERTAINTY OF MEASUREMENT FOR TES-AC-203, TOTAL AND INSOLUBLE DIETARY FIBRE (AOAC PROCEDURE)

Date of report : 03-11-09
Prepared by : A.N. Other

N.B. Explanations, which are not part of the report have outlined boxes

1.   Measurand specification

1.1   Scope
The AOAC procedure for analysing Total Dietary Fibre (TDF). The results apply to the sub-samples taken from the sample supplied by the client. The method is applicable to general foodstuffs.

1.2   Method
Two sub-samples (dried if necessary, defatted if containing over 10% fat, and de-sugared if containing over 50% sugar) are enzymatically digested to remove protein and starch. After precipitation of soluble dietary fibre, the total residue is dried and weighed. One sub-sample is analysed for protein and the other for ash. Total Dietary Fibre (TDF) is calculated as the weight of residue minus the weight of protein plus ash in the residue.

1.3   Equation

$$\text{TDF}\ (\%) = \frac{(\text{Rmw - Pw - Aw - B})}{\text{Sw (mean)}}\ \text{x}\ 100$$

Where :
Rmw = Mean weight in mg of two dried subsample residues
Pw = Weight in mg of protein
Aw = Weight in mg of Ash
B = Residue, Protein, and Ash weights for blank sample calculated as Blank
fibre value, mg B = Rmwb – Pwb – Awb
Sw = mean weight of two sub-samples

2.   Identification of Uncertainty Sources
Uncertainty sources are recorded in the flow diagram in **Box 2**. UoM-1-TES-AC-203-12-AOAC-TDF.xlsx[c]. All known sources of measurement uncertainty have been accounted for.

3.   Quantification of Uncertainty

3.1   Simplification by grouping sources covered by existing data
Sources of uncertainty identified in **Box 2** are accounted for within the following QC data :

---

[c]The file containing the UoM assessment should have a name which uniquely identifies it,, and enables specific assessments to be found easily. The filename UoM-1-TES-AC-203-12-AOAC-TDF.xlsx signifies that it is the first assessment of the unique method code of which it is the 12th edition.

- Duplicate analyses of one of a series of in-house reference material were determined with each batch of samples (see Box 3 for further details of the material analysed). Results must fall within defined limits as recorded in the Quality Control records for the method.
- Three analysts have generated the results used in the data set used to calculate uncertainty

3.2   Quantification of grouped components

Precision and bias are estimated from the data sources noted in **Box 3** and the results referred to in **Box 5**.

3.3   Quantification of remaining components

It is considered that the data provided for the calculation of uncertainty covers all known components.

3.4   Conversion of components to standard deviations

The required conversion to standard deviations is provided in the in-house worksheet referred to in the main text.

4.   Calculation of the Combined Uncertainty

4.1   Calculation of the Combined Uncertainty

The precision and bias calculated in **Box 5**, provide the combined relative uncertainty. The charted precision and bias data (**Box 4**) indicate that a 'relative uncertainty' result is most appropriate (0.15). The relative bias was negligible so no additional allowance need be made for bias and its uncertainty.

4.2   Calculation of the expanded uncertainty

The calculation of expanded uncertainty is provided from **Box 5**. The relative expanded uncertainty is 0.151; i.e. 15.1% of the result.

Calculate the expanded uncertainty in the same units as the result by multiplying the result by 0.151; e.g. if the result is 11.61 %w/w TDF, expanded uncertainty = 11.61 x 0.151 = 1.753 %w/w TDF.

5.   Reporting uncertainty

1) **Report the result and uncertainty in the form 'result ± uncertainty'**
Report the uncertainty to 2 *significant figures* (e.g. 1.753% as 1.8%)
Report the result to the same number of *decimal places* as the uncertainty.

Sample D result = 11.6 %w/w ± 1.8 %w/w TDF[d]

The result and its uncertainty are reported in the above simple form. A footnote[d] is provided to show how the uncertainty of measurement is derived.

---

[d] The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor of k=1.97, providing a level of confidence of approximately 95%.

6.   Review

The evaluation of measurement uncertainty shall be reviewed and verified if the method is altered in any significant way. As new information, such as internal and external quality assessment results become available, it will be compared with the estimate Expanded Uncertainty and, if it is incompatible the reason will be investigated. In any case, the evaluation of measurement uncertainty will be reviewed and verified before the end of November 2012.

6.1   Date of assessment and the Assessor
03-11-09 A.N. Other

6.2   Date for Review
02/11/12

7.   File storage

Unique filenames given as an example:

>   Calculations: UoM-1-TES-AC-203-12-AOAC-TDF.xlsx
>   Report: UoM-1-TES-AC-203-12-AOAC-TDF.docx

stored in the chemistry fileshare under "Uncertainty of measurement/UoM-record-archive"

APPENDIX II   THE EQUATIONS EMPLOYED TO OBTAIN THE EXPRESSIONS NOTED IN THE UOM WORKSHEET

1 Precision

a) Given a data set x_{ij}

    where: $i = 1{:}n$ indicates the sample which has r_i replicates (r_i may $= 1$)

                $j = 1{:}r_i$ indicates the replicate of the ith sample

    then

b) total number of data $= N = \sum_{i=n} r_i$

c) mean of sample $i = \bar{x}_{i\cdot} = \dfrac{\sum_{j=1:r_i} x_{ij}}{r_i}$

(1.1) Precision "degrees of freedom"

precision degrees of freedom $= df_{prec} = N - n$

(1.2) "Precision s.d" (absolute)

a) define "absolute deviation" of each result from the mean for the sample as $\left(x_{ij} - \bar{x}_{i\cdot}\right)$

b) then "absolute" precision sd = root mean square(absolute deviation from mean) =

$$s_{prec.absolute} = \sqrt{\frac{\sum_{i=1:n}\left(x_{ij} - \bar{x}_{i\cdot}\right)^2}{df_{prec}}}$$

(1.3) "Precision (relative)"

a) define "relative deviation" of each result from the mean for the sample as $\dfrac{\left(x_{ij} - \bar{x}_{i\cdot}\right)}{\bar{x}_{i\cdot}}$

b) then "relative" precision sd = root mean square(relative deviation from mean)

$$= s_{prec.relative} = \sqrt{\frac{\sum_{i=1:n}\left(\dfrac{x_{ij} - \bar{x}_{i\cdot}}{\bar{x}_{i\cdot}}\right)^2}{df_{prec}}}$$

2 Bias

Given a data set of n pairs of values $\{x_i, y_i\}$, $i=1:n$
where x_i = observed value
y_i = true value

(2.1) "degrees of freedom"

degrees of freedom associated with bias estimate $= df_{bias} = n-1$

(2.2) "Bias" (absolute)

a) define "absolute error" of each pair as $\left(x_i - y_i\right)$

b) then "absolute" bias = mean(absolute error) $= bias_{absolute} = \dfrac{\sum\limits_{i=1:n}\left(x_i - y_i\right)}{n}$

(2.3) "s.e. Bias" (absolute)

a) standard deviation of absolute errors $= s_{bias.absolute} = \sqrt{\dfrac{\sum\limits_{i=1:n}\left(\left(x_i - y_i\right) - bias_{absolute}\right)^2}{df_{bias}}}$

b) standard error of absolute bias $= s.e._{bias.absolute} = \dfrac{s_{bias.absolute}}{\sqrt{n}}$

(2.4) "Bias (relative)"

a) define "relative error" of each pair as $\dfrac{\left(x_i - y_i\right)}{y_i}$

b) then "relative" bias = mean(relative error) $= bias_{relative} = \dfrac{\sum\limits_{i=1:n}\dfrac{\left(x_i - y_i\right)}{y_i}}{n}$

(2.5) "s.e. Bias (relative)"

a) standard deviation of relative errors $= s_{bias.relative} = \sqrt{\dfrac{\sum\limits_{i=1:n}\left(\dfrac{\left(x_i - y_i\right)}{y_i} - bias_{relative}\right)^2}{df_{bias}}}$

b) standard error of relative bias $= s.e._{bias.relative} = \dfrac{s_{bias.relative}}{\sqrt{n}}$

3 Combined Absolute|Relative Uncertainty

(3.1) "standard uncertainty"

	Absolute	Relative
Ignore	$u = \sqrt{s_{prec.absolute}^2}$	$u = \sqrt{s_{prec.relative}^2}$
Include	$u = \sqrt{s_{prec.absolute}^2 + s.e._{bias.absolute}^2}$	$u = \sqrt{s_{prec.relative}^2 + s.e._{bias.relative}^2}$

(3.2) "degrees of freedom"

	Absolute	Relative
Ignore	$df_{unc} = \dfrac{u^4}{\left(\dfrac{s_{prec.absolute}^4}{df_{prec}}\right)}$	$df_{unc} = \dfrac{u^4}{\left(\dfrac{s_{prec.relative}^4}{df_{prec}}\right)}$
Include	$df_{unc} = \dfrac{u^4}{\left(\dfrac{s_{prec.absolute}^4}{df_{prec}} + \dfrac{s.e._{bias.absolute}^4}{df_{bias}}\right)}$	$df_{unc} = \dfrac{u^4}{\left(\dfrac{s_{prec.relative}^4}{df_{prec}} + \dfrac{s.e._{bias.relative}^4}{df_{bias}}\right)}$

(3.3) "coverage factor (k)"

$k = t_{df_{unc}}^{-1}(1 - conf)$

where t_n^{-1} = inverse of the Students-t distribution with n degrees of freedom

$conf$ = desired confidence

(3.4) "expanded uncertainty"

$U = ku$

(3.5) "Bias is [NOT] negligible"

IF	Absolute	$\left(bias_{absolute} < \dfrac{s_{prec.absolute}}{5}\right)$ and $\left(s.e._{bias.absolute} < \dfrac{s_{prec.absolute}}{5}\right)$	One cell = "Bias is negligible" and a further cell= blank
	Relative	$\left(bias_{relative} < \dfrac{s_{prec.relative}}{5}\right)$ and $\left(s.e._{bias.relative} < \dfrac{s_{prec.relative}}{5}\right)$	
	ELSE ="Bias is NOT negligible" and ="CONSULT YOUR LINE MANAGER"		

(3.6) " Absolute|Relative bias"

Only shown if ="Bias is NOT negligible" AND ="Ignore"

="Absolute"=$bias_{absolute}$;   ="Relative" =$bias_{relative}$

(3.7) "Expanded uncertainty" on bias

Only shown if ="Bias is NOT negligible" AND ="Ignore"

="Absolute"=$s.e._{bias.absolute}\, t_{df_{bias}}^{-1}(1 - conf)$;   ="Relative"=$s.e._{bias.relative}\, t_{df_{bias}}^{-1}(1 - conf)$

where t_n^{-1} = inverse of the Students-t distribution with n degrees of freedom

$conf$ = desired confidence