



A White Paper from FOSS

Measurement uncertainty: “Top down” or “Bottom up” ?

By Dr. Jürgen Möller

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Based on a scientific understanding of the theoretical principles of a method together with practical experiences, an estimation of the uncertainty of a measurement must be made (1).

The EURACHEM / CITAC Guide “Quantifying Uncertainty in Analytical Measurement” (2) describes two approaches:

A: The step-by-step or component-by-component approach (bottom up)

B: The use of collaborative trial data (top down).

The top down approach

The bottom up approach is usually preferred for well-defined metrological measurands, whilst it is common to use a top down approach for empirical methods like moisture, ash, fat, fibre and crude protein, where the result is method-dependent. The top down approach is based on using the performance parameters of collaboratively studied reference methods.

If a laboratory is using validated standard methods, it has only to prove that it is capable of applying these methods within their repeatability and reproducibility limits. This can be done by participating in a proficiency testing scheme.

The reproducibility limit R can directly be used as expanded measurement uncertainty U (expansion factor $k = 2,8$, sR = standard deviation of reproducibility) (3):

$$U = R = 2,8 * sR$$

Examples of international standard methods that may be used include:

EN ISO 5983-2:2005 Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content - Part 2: Block digestion/steam distillation method

AOAC 2001.11 Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds

EN ISO 20483:2006 Cereals and pulses – Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method, and the ISO 8968 / IDF 20 series (parts 1 – 5) for the determination of nitrogen in milk.

The top down approach is very practical for routine purposes but does not take systematic errors into account. You may think this is of no consequence, provided that everyone

uses the same method with the same known bias, but a correction for systematic errors is often required - not only when using different methods but also to address variations in blank values; contaminations; incomplete recoveries etc.

ISO 17025 assessors would like a more detailed overview of any error sources involved, in the form of a fishbone diagram at the very least. The same applies to the development of methods and instruments.

The bottom up approach

Normally the error structure must be described using a more complex model. The most common measurement errors should be segmented according to the different stages of the procedure, including the measurement equipment, performance, conditions of measurement and/or the measurement object itself.

The bottom up approach demands a clear description of what is being measured, including the relationship between the analyte and the parameters upon which it depends. Usually this is achieved using a flowchart of steps in the measurement procedure, and by providing the formula for calculating the result.

For Kjeldahl analysis, the formula is:

$$w_N = \frac{1,4007(V_s - V_b)c_s}{m}$$

V_s is the numerical value of the volume of the hydrochloric acid standard volumetric solution used in the determination (mL).

V_b is the numerical value of the volume of the hydrochloric acid standard volumetric solution used in the blank test (mL).

c_s is the numerical value of the exact concentration, in moles per litre, of the hydrochloric acid standard volumetric solution, expressed to four decimal places.

m is the numerical value of the mass of the test portion in grams.

The main steps in the determination of protein/nitrogen are sampling/sample preparation, digestion and distillation/titration. Each has an influence on the measurement uncertainty for the determination of crude protein.

Sampling always makes a significant contribution to the overall measurement uncertainty, yet it is often not included in the calculation. The total sampling error is by far the dominating contribution to all analytical endeavours; often 100 times larger than the total analytical error. Just imagine that in one ear of wheat, the protein content may vary between 7 % and 20%! The same would apply to a whole field, depending on the growing conditions.

Even if you apply a proper composite sampling strategy, you will end up with a 1 kg or 10 kg lab sample that must be subdivided and prepared before a final test sample of 1 g fine flour can be obtained. Standard methods most frequently state that sampling is not part of this method, and in collaborative studies performed for the validation of these methods, only well homogenised flours are distributed. Quantifying the measurement uncertainty of sampling is not an easy task. For more information, please follow the link below (7).

Sampling also includes sample preparation, where the sub-division and grinding/ homogenisation of laboratory samples has an influence on the measurement uncertainty.

Steps in the digestion procedure, and factors influencing measurement uncertainty include the weighing of samples into digestion tubes (calibration of balance, uncertainty in weighing); the addition of 12 – 20 ml concentrated sulphuric acid (purity, uncertainty of added volume); the addition of potassium sulphate and a catalyst (effect of salt concentration and catalyst), and the digestion temperature and time (significance of errors in time and temperature). All these effects are described in "A Handbook for Kjeldahl Digestion", by Jan-Åke Persson (This is available from Foss)(6).

It is well known that in Kjeldahl determinations, Mercury is the most effective catalyst. When using other catalysts, the digestion conditions - especially the digestion time - must be changed to produce acceptable recoveries. This leads to longer digestion times and lower sample throughputs.

The use of amino acids for recovery studies may not always be the best choice, as they are not always available in known purities and do not reflect the composition of the sample. In many cases it may be better to use real samples of known composition (CRM's) and/or participate in proficiency testing schemes (PTS). In practice, digestion times are optimised to reach 98-99 recoveries on actual samples.

Kjeldahl is very good at recovering alfa amino nitrogen existing in protein, but has problems with heterocyclic nitrogen existing in nicotinic acid. Figure 1 shows the recoveries for different positions in a Tecator digestion system (first two positions = blank). This is a good way to characterise the performance of the digestive system. Centre positions offer slightly better recovery than outer positions.

The standard deviation obtained can directly be used for calculating measurement uncertainty. You must also consider the bias, i.e. the deviation from 100% recovery.

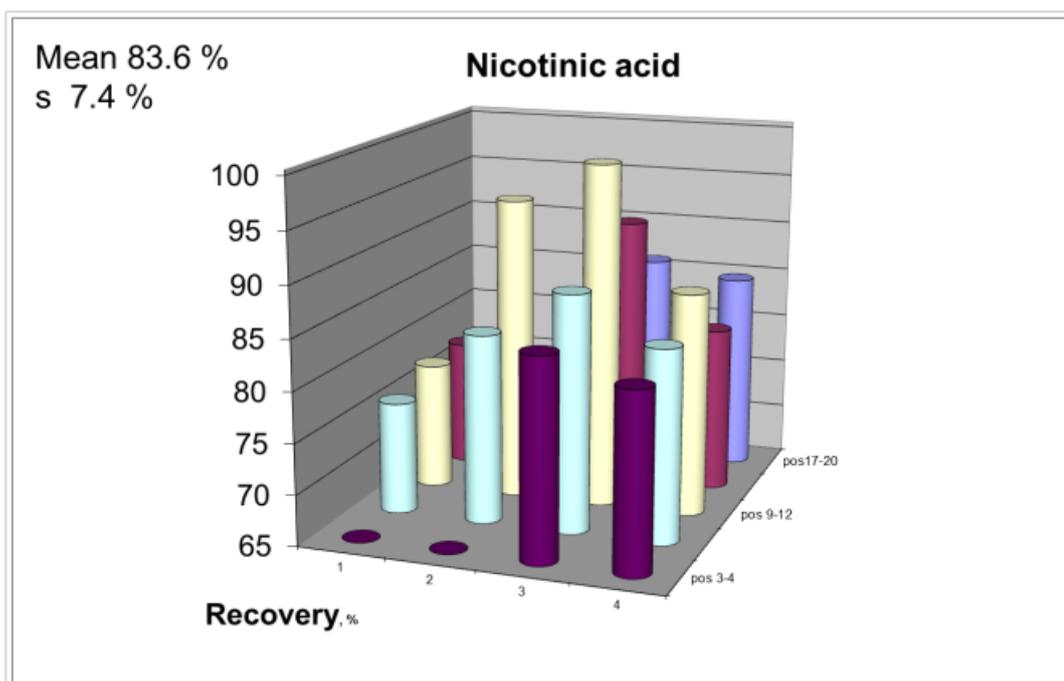


Figure 1 (see text)

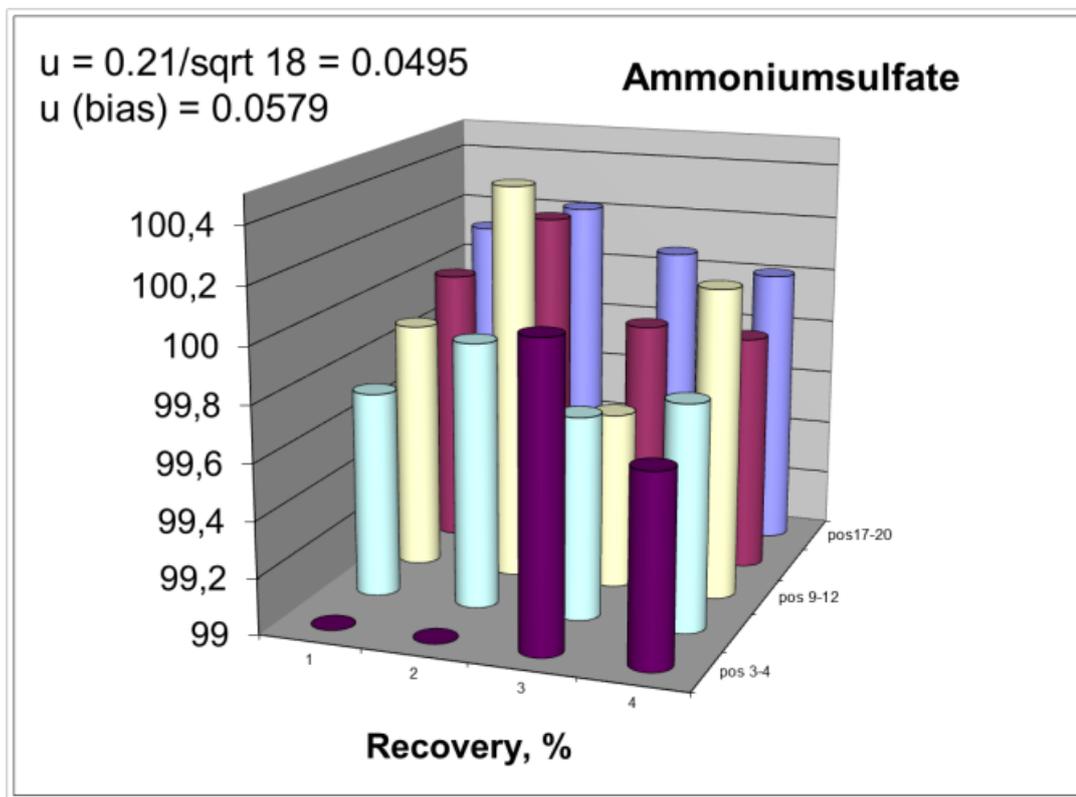


Figure 2 (see text)

Another example is the digestion of ammonium sulphate (see figure 2). Please observe the scale from 99.0% to 100.4%.

In this case, only insignificant losses and/or contamination have occurred. A view of the total uncertainty for a homogeneous sample is provided, together with the subsequent distillation and titration steps.

While some of the sources in the measurement procedure can be quantified and calculated, others are more difficult; hence estimations must be made.

You can for instance use ammonium solutions of known concentrations and determine the mean and standard deviation for the titration, distillation + titration, and distillation + titration + digestion steps. The differences will allow you to determine their respective influence.

In distillation the addition of steam; the steam effect; addition of sodium hydroxide; transport and capture of generated ammonia; design of splash head; temperature of condensate and receiver solution all have a role to play.

In titration the concentration, volume and temperature of the titrant all play a role. Furthermore, the adjustment of the photometric end-point may lead to a systematic error. Regarding measurement uncertainty for the titration step, you can find a comprehensive example in (2), which includes the uncertainty of the differential weighing of samples (mass m) and the concentration of the hydrochloric acid used for titration. Other comprehensively elaborated examples for the Kjeldahl determination can be found under references (4, 5).

The effect of temperature on the dosed volume is sometimes underestimated. Estimations are based on a variation of 1-2 C of the lab atmosphere only. This may not be sufficient. In some instruments the burette is placed close to the steam generator and/or splash head, with either zero or poor heat insulation, resulting in considerable temperature differences.

After identification of all sources that may contribute to measurement uncertainty, and estimation of their relative contribution, the combined relative standard uncertainty can be calculated.

$$u_r(X) = \sqrt{u^2(V-V_0) + u^2(C_{HCl}) + u^2(m) + u^2(REP)}$$

Uncertainty component	AcQuAs s (3)	In Focus (4)	Eur guide (5)
u_r mass m	0,00113	0,00090	0,00050
u_r c (HCl)	0,00110	0,00130	0,00018
u_r 14,007	ins ignificant		0,00003
u_r (Vs - Vo)	0,00177	0,00117	0,00111
u_r total rep.	0,00000	0,00104	0,00128
u_x total	0,00237	0,00222	0,00178

The above table shows three examples of relative, standard uncertainties that have been combined into one overall uncertainty. (3) is an example for insulin, (4) is for soy beans and (5) is for barley, based on the Eurachem guidelines (see also respective references).

Not all examples are based on the same assumptions/estimations and not all have incorporated a total repeatability term; they produce similar results (the same order of magnitude, at least) for the total standard uncertainty.

Conclusions:

The bottom up (step by step) approach for estimating measurement uncertainty gives valuable insight into different sources of errors, thus indicating potential improvements. But results may vary depending on the estimations made.

The top down approach - using performance data from collaboratively studied and validated standard methods - may result in higher measurement uncertainties, as it is based on reproducibility data. But it does allow parameter/method-specific "uniform" measurement uncertainty values.

ISO 17025 states that in cases where this rigorous approach cannot be applied, the laboratory must at least attempt to identify all the components of uncertainty and make a reasonable estimation. They must also ensure that the form of reporting of the result does not create a wrong impression of the uncertainty.

Reasonable estimation must be based on knowledge of the performance of the method and on the measurement scope. It should also utilise previous experience and validation data, for example.

The conclusion is therefore, that a top down approach that is complemented with an estimation of error sources would be a good solution.

References:

1. ISO/IEC 17025:2005 – <http://www.iso.org>
2. www.measurementuncertainty.org
3. ISO/TS 21748:2004 – <http://www.iso.org>
4. Uncertainty of...Kjeldahl method (Thomas Anglov, Inge M. Petersen, Jesper Kristiansson, Accred. Qual. Assur., 1999, 4:505-510)
5. Measurement uncertainty (C. Shuwei et al., In Focus 32(1), 2008, p 24-27), www.foss.dk
6. Handbook for Kjeldahl digestion, www.foss.dk
7. <http://www.nordicinnovation.net/nordtestfiler/tr604.pdf>

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