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EVALUATION OF MEASUREMENT UNCERTAINTY
ANNEX 1.2

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Annex 1 to Guideline "Evaluation of Measurement Uncertainty"
PA/PH/OMCL (18) 145 (in its current version)

Estimation of measurement uncertainty of using Bottom-up approach

Annex 1.2 Estimation of Uncertainty of measurement for quantitative determination using spectrophotometric method (calibration curve method)

Example: Determination of $[NH_4]^+$ in Water

1. Description of the method

Determination of NH_4^+ in water (a parameter listed in the specification of the purified water) is performed using a dye which forms a photometric complex with ammonium. The absorbance of the ammonium-dye complex is measured at 640-660 nm.

The concentration of ammonium ($C_{\mathrm{NH}_4^+}$) is calculated from the measured absorbance of the sample solution, using calibration curve (absorbance *vs* concentration of standard solutions).

Preparation of sample solution:
20.0 mL of the sample to be analysed is diluted to 25.0 ml by the chromophore solution.

Calibration curve:
Calibration curve was constructed from the relationship between absorbance and concentration of standard solutions at 5 different concentrations (0.050 mg/L, 0.100 mg/L, 0.200 mg/L, 0.250 mg/L and 0.500 mg/L). The least squares approach was applied to check the linear relationship between calibration data pairs (x_i, y_i).

2. Estimation of the measurement uncertainty

Approach 1

2.1 Step 1. Specification of a measurand

The output quantity ($C_{\mathrm{NH}_4^+}$) is calculated by means of the following function [4]:

$$(C_{\mathrm{NH}_4^+}) = \left[\frac{A_{NH4} - a}{b}\right] \cdot \frac{20}{25} + \Delta C_{dc}$$

Where:
A_{NH4}: measured absorbance of the sample solution;
a and b: intercept and slope of the calibration curve ($y = a + bx$)
$\frac{20}{25}$: dilution factor, f_d
ΔC_{dc}: Component taking into account impurities

2.2 Step 2. Identification of Uncertainty Sources

Table 1. Uncertainty sources and components

Input variable	**Uncertainty components**	***u*(*x*_i)**
A_{NH4}	Interferences Repeatability Drift of photometer	$u(A_{NH4})$
a and *b*	Purity of standard material Preparation of solutions Repeatability and drift of photometer	$u(a)$ $u(b)$
f_d	Preparation of sample solution	$u(fd)$
ΔC_{dc}	Contamination Decomposition Volatilization Incomplete reaction	$u(\Delta C_{dc})$

Each of the parameters that affect the value of the measurand are shown as a cause and effect diagram (Fig. 1) [1, 2].

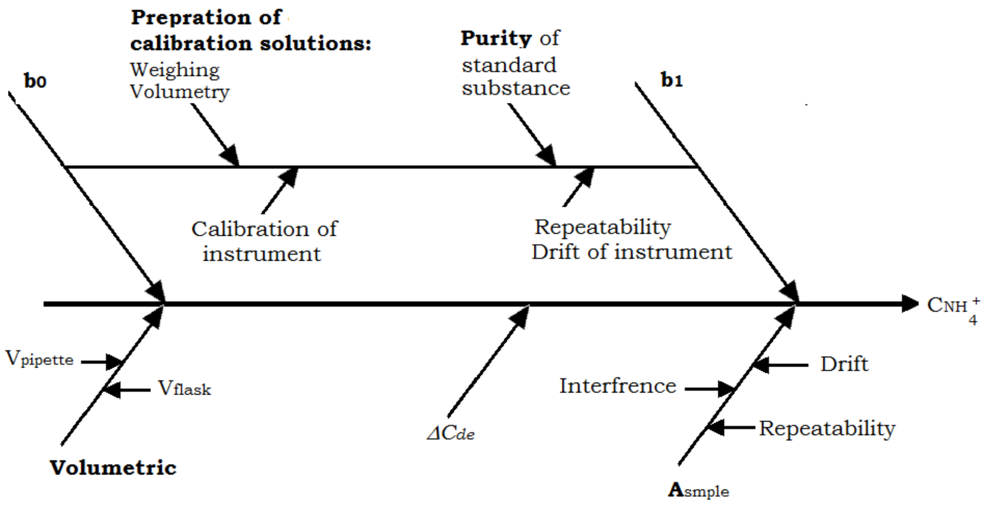


Fig. 1 Cause and effect diagram for determination of $C_{\mathrm{NH}_4^+}$

2.3 Step 3. Quantification of uncertainty components

2.3.1 Component 1: Uncertainty of preparation of the sample

Uncertainty of preparation of the sample is associated with the uncertainty of the dilution factor, calculated as:

$$fd = \frac{V_2}{V_1} = \frac{25mL}{20\,mL} = 1.25$$

The standard uncertainty of the dilution factor $u(fd)$ is:

$$\frac{u(fd)}{fd} = \sqrt{(\frac{u(V_1)}{V_1})^2 + (\frac{u(V_2)}{V_2})^2}$$

The standard uncertainties $u(V_i)$ are calculated as:

$$u(V_i) = \sqrt{u(Vcal)^2 + u(Vrep)^2 + u(VT)^2}$$

Table 2. Uncertainty values of the dilution volume (calculation of these values is explained in Annex 1.1)

mL	Volume	$u(VT)$	$u(Vrep)$	$u(Vcal)$	$u(V_i)$	$\frac{u(V_i)}{V_i}$
V_1	20 mL	0.0097 mL	0.0083 mL	0.01633 mL	0.0249 mL	0.001243
V_2	25 mL	0.01212 mL	0.0125 mL	0.01225 mL	0.0273 mL	0.001093

$$\frac{u(fd)}{fd} = \sqrt{(0.001243)^2 + (0.001093)^2} = 0.00134$$

$$u(fd) = 0.00134 \cdot 1.25 = 0.001677$$

2.3.2 Component 2: Uncertainty of the absorbance of the sample solution

Uncertainty of the absorbance $u(A_{NH4})$

Table 3. Values of the uncertainties related to $u(A_{NH4})$

Description	**Value**	**Unit**
$u(A_{NH4}repeatability)$	0.0012	AU
$u(A_{NH4}drift)$	0.0014	AU
$u(A_{NH4}interference)$	0.0025	AU

$$u(A_{NH4}) = \sqrt{u(A_{NH4}repeatability)^2 + u(A_{NH4}drift)^2 + u(A_{NH4}interference)^2}$$

$$u(A_{NH4}) = \sqrt{0.0012^2 + 0.0014^2 + 0.0025^2} = 0.003106\ \mathrm{AU}$$

Table 4. Values of the uncertainties related to absorbance of the sample solution

Description	**Value**	**Unit**
$u(A_{NH4})$	0.00311	AU
$u(\Delta C_{dc})$	0.002*	mg/mL

*The uncertainty of ΔC_{dc} is established based on experience to 0.002 mg/mL.

2.3.3 Component 3 Uncertainty of the slope and intercept of the calibration curve

There are four main sources of uncertainty to consider when estimating uncertainty of the estimated concentration x_{pred}.

1. Random variations in absorbance (both reference y_i and measured y_{obs}).
2. Random effects in assigned reference value, x_i.
3. Constant unknown offset on x_i and y_i.
4. The assumption of linearity may not be valid [3].

However, the most significant of these sources is the first source, i.e. random variations in measurement of y_i and y_{obs}, which are estimated from the residual standard deviation S [1, 4].

$$S(residual) = \sqrt{\frac{\sum_{j=1}^{n}[Ai - (a + b \cdot c_i)]^2}{n-2}}$$

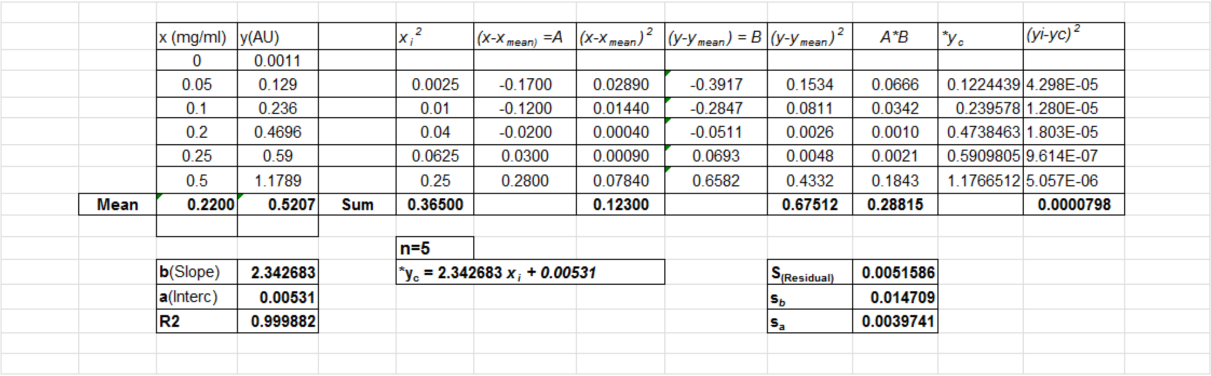
$$S(residual) = \sqrt{\frac{\sum_{i=0}^{n}[y_i - y_c]^2}{n-2}}$$

Estimation of the uncertainties was performed by the following equations:

$$S(b) = \frac{S(residual)}{\sqrt{\sum(xi - \bar{x})^2}}$$

$$S(a) = S(residual) \cdot \sqrt{\left[\frac{\sum(xi^2)}{n\sum(xi - \bar{x})^2}\right]}$$

Table 5. Calibration results



	x (mg/ml)	y(AU)		x_i^2	$(x-x_{mean}) = A$	$(x-x_{mean})^2$	$(y-y_{mean}) = B$	$(y-y_{mean})^2$	A*B	$^*y_c$	$(yi-yc)^2$
	0	0.0011									
	0.05	0.129		0.0025	-0.1700	0.02890	-0.3917	0.1534	0.0666	0.1224439	4.298E-05
	0.1	0.236		0.01	-0.1200	0.01440	-0.2847	0.0811	0.0342	0.239578	1.280E-05
	0.2	0.4696		0.04	-0.0200	0.00040	-0.0511	0.0026	0.0010	0.4738463	1.803E-05
	0.25	0.59		0.0625	0.0300	0.00090	0.0693	0.0048	0.0021	0.5909805	9.614E-07
	0.5	1.1789		0.25	0.2800	0.07840	0.6582	0.4332	0.1843	1.1766512	5.057E-06
Mean	**0.2200**	**0.5207**	**Sum**	**0.36500**		**0.12300**		**0.67512**	**0.28815**		**0.0000798**

		n=5		
b(Slope)	**2.342683**	$^*y_c = 2.342683\, x_i + 0.00531$	$S_{(Residual)}$	**0.0051586**
a(Interc)	**0.00531**		s_b	**0.014709**
R2	**0.999882**		s_a	**0.0039741**

Therefore, $y = 0.00531 + 2.34268x$

Using this equation y_c can be calculated with known values for x and their corresponding square of difference, i.e. $[y_i - y_c]^2$.

$$S(residual) = \sqrt{\frac{\sum_{i=0}^{n}[y_i - y_c]^2}{n-2}} = \sqrt{\frac{7.9835 \cdot 10^{-5}}{3}} = 0.0051586\ \mathrm{AU}$$

$$S(b) = \frac{S(residual)}{\sqrt{\sum(xi - \bar{x})^2}} = \frac{0.0051586}{\sqrt{0.12300}} = 0.014709\ \mathrm{AU \cdot L/mg}$$

$$S(a) = S(residual) \cdot \sqrt{\left[\frac{\sum(xi)2}{n\sum(xi - \bar{x})^2}\right]} = 0.00477 \cdot \sqrt{\frac{0.365}{5 \cdot 0.1230}} = 0.0039741\ \mathrm{AU}$$

The standard uncertainty of *a* and *b* are approximation of the uncertainties. Because it neglects the systematic effects that impact all the points on the regression line. Furthermore, it neglects the correlation between *a* and *b*.

2.4 Step 4. Calculation of combined standard uncertainty and expanded uncertainty

The combined uncertainty is estimated by the following procedure, assuming that all variables are independent, i.e. there is no correlation between the measured values.

$$u_c = \sqrt{\left[\left(\frac{\delta C_{NH4}}{\delta A_{NH4}}\right)^2 \cdot u^2(A_{NH4}) + \left(\frac{\delta C_{NH4}}{\delta a}\right)^2 \cdot u^2(a) + \left(\frac{\delta C_{NH4}}{\delta b}\right)^2 \cdot u^2(b) + \left(\frac{\delta C_{NH4}}{\delta fd}\right)^2 \cdot u^2(fd) + \left(\frac{\delta C_{NH4}}{\delta \Delta Cdc}\right)^2 \cdot u^2(\Delta Cdc)\right]}$$

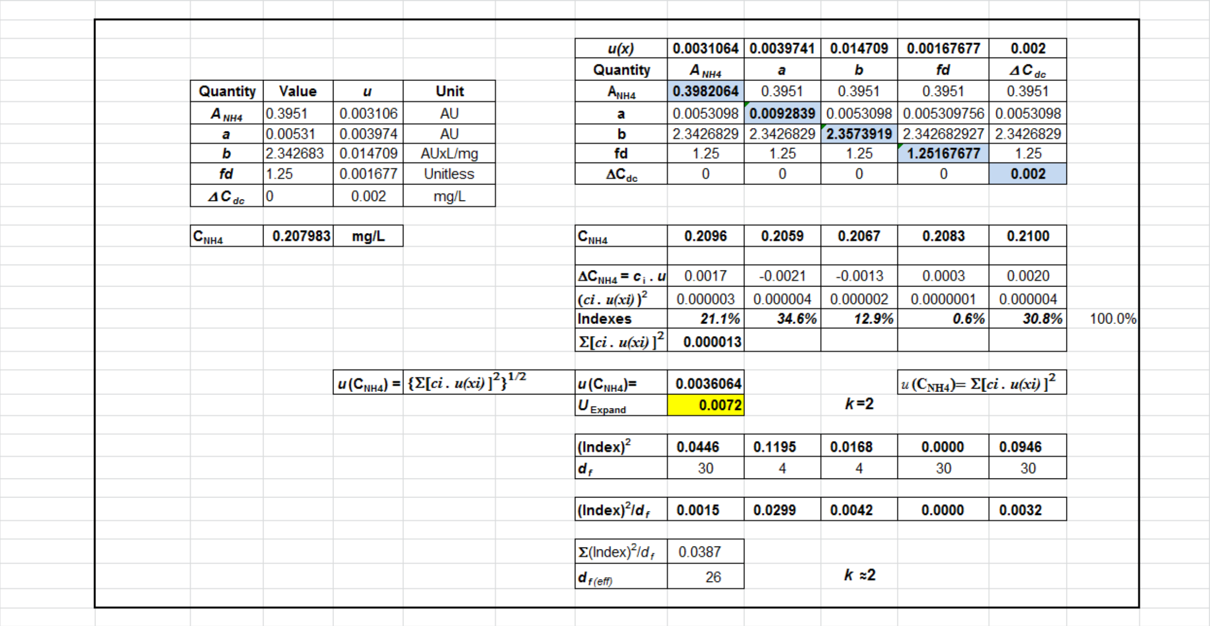
Replacing $\partial C_{sample} / \partial x_i$ by c_i the equation can be written as:

$$u_c = \sqrt{[c^2(A_{NH4}) \cdot u^2(A_{NH4}) + c^2(a) \cdot u^2(a) + c^2(b) \cdot u^2(b) + c^2(fd) \cdot u^2(fd) + u^2(\Delta Cdc) \cdot u^2(\Delta Cdc)]}$$

The partial derivatives ($\partial_y / \partial_{xi}$) or sensitivity coefficients (c_i) describes how the output estimate (y or C_{NH4}) varies with changes in the values of the input estimates [4].

$$u(y) = |c_i| \cdot u(x_i)$$

Table 6 Estimation of the combined uncertainty by the spread sheet approach



Quantity	Value	u	Unit
A_{NH4}	0.3951	0.003106	AU
a	0.00531	0.003974	AU
b	2.342683	0.014709	AUxL/mg
fd	1.25	0.001677	Unitless
ΔC_{dc}	0	0.002	mg/L

C_{NH4}	**0.207983**	**mg/L**

$u(x)$	0.0031064	0.0039741	0.014709	0.00167677	0.002	
Quantity	A_{NH4}	a	b	fd	ΔC_{dc}	
A_{NH4}	**0.3982064**	0.3951	0.3951	0.3951	0.3951	
a	0.0053098	**0.0092839**	0.0053098	0.005309756	0.0053098	
b	2.3426829	2.3426829	**2.3573919**	2.342682927	2.3426829	
fd	1.25	1.25	1.25	**1.25167677**	1.25	
ΔC_{dc}	0	0	0	0	**0.002**	
C_{NH4}	**0.2096**	**0.2059**	**0.2067**	**0.2083**	**0.2100**	
$\Delta C_{NH4} = c_i \cdot u$	0.0017	-0.0021	-0.0013	0.0003	0.0020	
$(ci \cdot u(xi))^2$	0.000003	0.000004	0.000002	0.0000001	0.000004	
Indexes	***21.1%***	***34.6%***	***12.9%***	***0.6%***	***30.8%***	100.0%
$\Sigma[ci \cdot u(xi)]^2$	**0.000013**					

$u(C_{NH4}) =$	$\{\Sigma[ci \cdot u(xi)]^2\}^{1/2}$	$u(C_{NH4})=$	**0.0036064**	$u(C_{NH4})= \Sigma[ci \cdot u(xi)]^2$
		U_{Expand}	**0.0072**	k=2

(Index)^2	**0.0446**	**0.1195**	**0.0168**	**0.0000**	**0.0946**
d_f	30	4	4	30	30
(Index)$^2/d_f$	**0.0015**	**0.0299**	**0.0042**	**0.0000**	**0.0032**

Σ(Index)$^2/d_f$	0.0387	
$d_{f(eff)}$	26	k ≈2

$$u(C_{NH4}) = \sqrt{[c_i{}^2 \cdot u^2(x_i)]} = \sqrt{\sum[(\Delta C_{NH4})^2]} = 0.0036\, mg/mL$$

Hence, $C_{\mathrm{NH_4^+}}$ = 0.208 ± 0.0072 mg/L (*k* = 2, approximately 95% level of confidence).

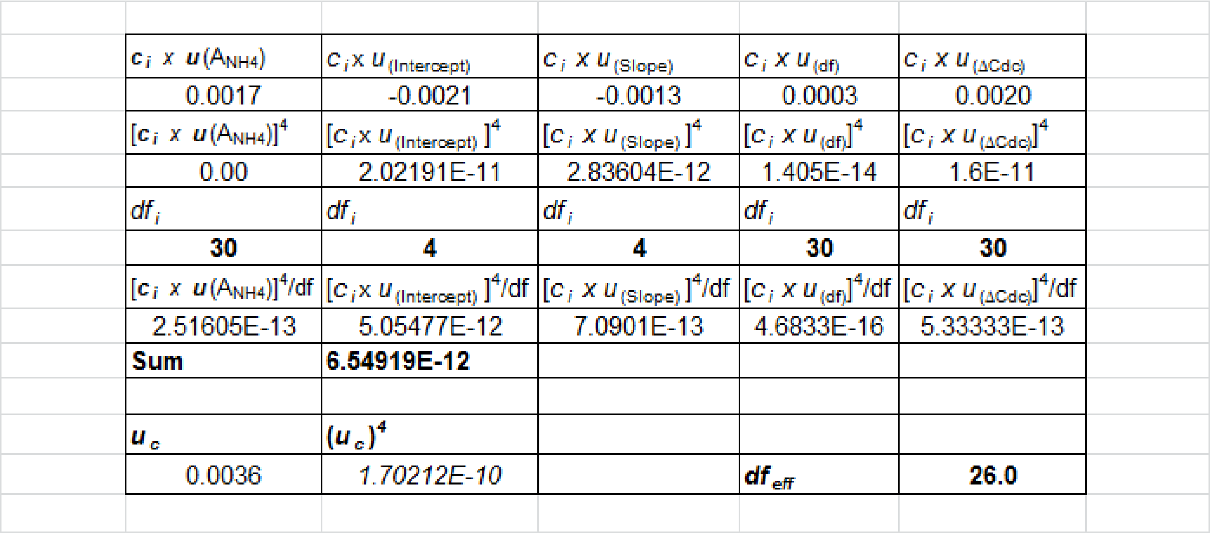
K = 2 is commonly used for calculation of expanded uncertainty. This value is appropriate when Type B uncertainty components dominate the uncertainty budget or when type A components have been established with ≥ 30 repeated measurements [5]. When Type A uncertainties dominate and where there are fewer than 30 degrees of freedom, a larger coverage factor is required [5]. The coverage factor in such cases is calculated by taking into account the effective degree of freedom (*df*$_{eff}$), which is calculated by either of the following equations [2, 5].

$$df_{eff} = \frac{1}{\sum \frac{Index_i^2}{df_i}}$$

The effective degree of freedom can also be calculated by the Welch-Satterthwaite formula [5]:

$$d_{eff} = \frac{u_c^4(y)}{\sum \frac{[c_i\, u(x_i)]^4}{df_i}}$$

Table 7 Calculation of the effective degree of freedom according to the Welch-Satterthwaite formula [5]



c_i x $u(A_{NH4})$	c_ix $u_{(Intercept)}$	c_i x $u_{(Slope)}$	c_i x $u_{(df)}$	c_i x $u_{(\Delta Cdc)}$
0.0017	-0.0021	-0.0013	0.0003	0.0020
$[c_i$ x $u(A_{NH4})]^4$	$[c_i$x $u_{(Intercept)}]^4$	$[c_i$ x $u_{(Slope)}]^4$	$[c_i$ x $u_{(df)}]^4$	$[c_i$ x $u_{(\Delta Cdc)}]^4$
0.00	2.02191E-11	2.83604E-12	1.405E-14	1.6E-11
df_i	df_i	df_i	df_i	df_i
30	**4**	**4**	**30**	**30**
$[c_i$ x $u(A_{NH4})]^4$/df	$[c_i$x $u_{(Intercept)}]^4$/df	$[c_i$ x $u_{(Slope)}]^4$/df	$[c_i$ x $u_{(df)}]^4$/df	$[c_i$ x $u_{(\Delta Cdc)}]^4$/df
2.51605E-13	5.05477E-12	7.0901E-13	4.6833E-16	5.33333E-13
Sum	**6.54919E-12**			
u_c	$(u_c)^4$			
0.0036	1.70212E-10		df_{eff}	**26.0**

$$u_c(C_{NH_4}) = \sqrt{\sum(\Delta C_{NH_4})^2} = 0.0036 \text{ mg/L}$$

Calculation of the output quantity

$$C_{\mathrm{NH_4^+}} = \left[\frac{A_{sample} - a}{b}\right] \cdot \frac{20}{25} + C_{dc} = \left[\frac{0.3951 - 0.00531}{2.342683}\right] \cdot 1.25 + 0 = 0.208 \text{ mg/L}$$

Hence, $C_{\mathrm{NH_4^+}} = 0.208 \pm 0.007 \text{ mg/l (k } = 2, \text{approximately } 95\% \text{ level of confidence)}$

The contribution of each uncertainty source to the combined uncertainty is depicted in the figure 2. As seen the dilution factor (f_d) is the minor uncertainty source.

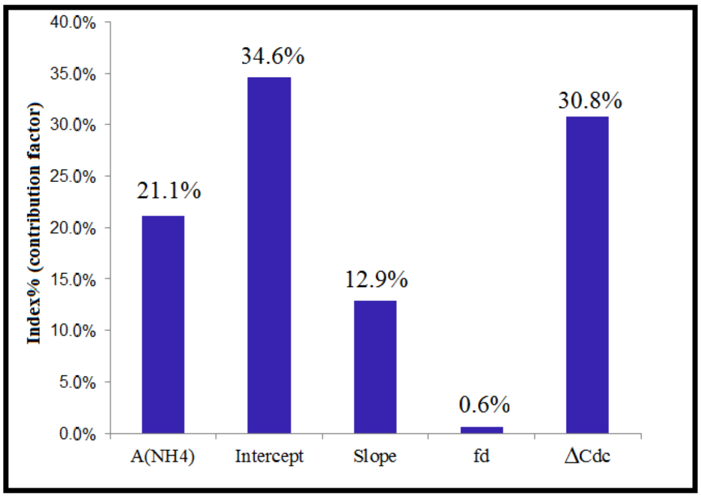


Fig. 2 Contribution of each uncertainty source to the combined uncertainty.

Approach 2

The purpose is to estimate the uncertainty for the determination of ammonium ions in water by taking into account the uncertainties in the reference standard solutions and the determined NH_4^+ concentration ($u(C_0)$ or $u(Cx_{obs})$) [3].

2.1 Step 1. Specification of a measurand

The output quantity ($C_{\mathrm{NH}_4^+}$) is calculated by means of the following function [2]:

$$(C_{\mathrm{NH}_4^+}) = c_0 \cdot fd + \Delta C_{dc}$$

Where:

$$c_0 = \left[\frac{A_{NH4} - a}{b}\right]$$

A_{NH4}: measured absorbance of the sample solution

a and b: intercept and slope of the calibration curve ($y = 0.00531 + 2.34268x$)

ΔC_{dc}: Component taking into account impurities

2.2 Step 2. Identification of uncertainty sources

Each of the parameters that affect the value of the measurand are shown as a cause and effect diagram (Fig. 1) [1,2].

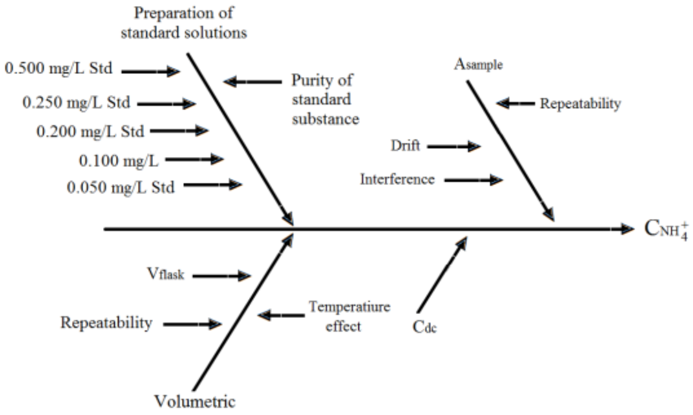


Fig. 1 Cause and effect diagram for determination of $C_{\mathrm{NH}_4^+}$

2.3 Step 3. Quantification of uncertainty components

2.3.1 Component 1: Uncertainty of preparation of the sample

Uncertainty of preparation of the sample is associated with the uncertainty of the dilution factor, calculated as:

$$fd = \frac{V_2}{V_1} = \frac{25mL}{20\ mL} = 1.25$$

The standard uncertainty of the dilution factor $u(fd)$ is:

$$\frac{u(fd)}{fd} = \sqrt{(\frac{u(V_1)}{V_1})^2 + (\frac{u(V_2)}{V_2})^2}$$

The standard uncertainties $u(V_i)$ are calculated as:

$$u(V_i) = \sqrt{u(Vcal)^2 + u(Vrep)^2 + u(VT)^2}$$

Table 2. Uncertainty values of the dilution volume (calculation of these values is explained in Anex 1.1)

mL	Volume	$u(VT)$	$u(Vrep)$	$u(Vcal)$	$u(V_i)$	$\frac{u(V_i)}{V_i}$
V_1	20 mL	0.0097 mL	0.0083 mL	0.01633 mL	0.0207 mL	0.00104
V_2	25 mL	0.01212 mL	0.0125 mL	0.01225 mL	0.0213 mL	0.00085

$$\frac{u(fd)}{fd} = \sqrt{(0.00104)^2 + (0.00085)^2} = 0.00134$$

$$u(fd) = 0.00166 \cdot 1.25 = 0.001677$$

2.3.2 Component 2 Preparation of reference standard solutions

The reference standard solutions were prepared from a 1.000 mg/L commercial solution, according to the following scheme. As there is no information regarding the level of confidence stated for the uncertainty of the stock solution, its uncertainty was disregarded.

Table 3. Preparation scheme for standard solutions

Sample	V_{Stock} (mL)	V_{Final} (mL)	Concentration, x_i (mg/L)
Blank	-	-	0
Ref Std 1	5	100	0.05
Ref Std 2	5	50	0.10
Ref Std 3	5	25	0.20
Ref Std 4	5	20	0.25
Ref Std 5	5	10	0.50

$$C_{final} = \frac{V_{stock\,solution} \cdot C_{stock\,solution}}{V_{final}}$$

The estimated standard uncertainties $u(V_i)$ are shown in Table 4.

Table 4. Uncertainty values of the dilution volume (calculation of these values is explained in Annex 1.1)

	Volume	$u(VT)\mathrm{mL}$	$u(Vrep)\mathrm{mL}$	$u(Vcal)\mathrm{mL}$	$u(V_i)$ mL	$\frac{u(V_i)}{V_i}$
V_1	5 mL	0.0024	0.0010	0.01633	0.0165	0.00331
V_2	10 mL	0.00485	0.0021	0.01633	0.0172	0.00172
V_3	20 mL	0.00970	0.0083	0.01633	0.0207	0.00104
V_4	25 mL	0.1212	0.0125	0.01225	0.0213	0.00085
V_5	50 mL	0.02425	0.0128	0.03266	0.0426	0.00085
V_6	100 mL	0.04850	0.0333	0.0408	0.0716	0.00072

The uncertainties of the reference standard solutions are summarised in tables 5-9.

Table 5. Uncertainty values of the dilution volume of the first reference solution

Uncertainty component	Quantity	Uncertainty	Relative uncertainty
V_{Stock}	5 mL	0.0165 mL	0.00331
V_{Final}	100 mL	0.0716 mL	0.00072

The concentration of the first standard solution is calculated as:

$$C_{ref1} = \frac{5\,mL \cdot 1.000\,mg/mL}{100\,mL} = 0.050\,mg/mL$$

The uncertainty of the concentration of the first reference solution is:

$$\frac{u(C_{ref1})}{C_{ref1}} = \sqrt{(0.00331)^2 + (0.00072)^2} = 0.00338$$

$$u\left(C_{ref1}\right) = 0.00338\ \cdot 0.05 = 0.000169\,mg/mL$$

Table 6. Uncertainty values of the dilution volume of the second reference solution

Uncertainty component	Quantity	Uncertainty	Relative uncertainty
V_{Stock}	5 mL	0.0165 mL	0.00331
V_{Final}	50 mL	0.0426 mL	0.00085

The concentration of the second standard solution is calculated as:

$$C_{ref2} = \frac{5\,mL \cdot 1.000\,mg/mL}{50\,mL} = 0.100\,mg/mL$$

The uncertainty of the concentration of the second reference solution is:

$$\frac{u(C_{ref2})}{C_{ref2}} = \sqrt{(0.00331)^2 + (0.00085)^2} = 0.00342$$

$$u\left(C_{ref2}\right) = 0.00342\ \cdot 0.100 = 0.000342\,mg/mL$$

Table 7. Uncertainty values of the dilution volume of the third reference solution

Uncertainty component	Quantity	Uncertainty	Relative uncertainty
V_{Stock}	5 mL	0.0165 mL	0.00331
V_{Final}	25 mL	0.0213 mL	0.00085

The concentration of the third standard solution is calculated as:

$$C_{ref3} = \frac{5\ mL \cdot 1.000\ mg/mL}{25\ mL} = 0.200\ mg/mL$$

The uncertainty of the concentration of the third reference solution is:

$$\frac{u(C_{ref3})}{C_{ref3}} = \sqrt{(0.00331)^2 + (0.00085)^2} = 0.003416$$

$$u\left(C_{ref3}\right) = 0.003416\ \cdot 0.200 = 0.000683\ mg/mL$$

Table 8. Uncertainty values of the dilution volume of the fourth reference solution

Uncertainty component	Quantity	Uncertainty	Relative uncertainty
V_{Stock}	5 mL	0.0165 mL	0.00331
V_{Final}	20 mL	0.0207 mL	0.00104

The concentration of the fourth standard solution is calculated as:

$$C_{ref4} = \frac{5\ mL \cdot 1.000\ mg/mL}{20\ mL} = 0.250\ mg/mL$$

The uncertainty of the concentration of the fourth reference solution is:

$$\frac{u(C_{ref4})}{C_{ref4}} = \sqrt{(0.00331)^2 + (0.00104)^2} = 0.003466$$

$$u\left(C_{ref4}\right) = 0.003466\ \cdot 0.250 = 0.000867\ mg/mL$$

Table 9. Uncertainty values of the dilution volume of the fifth reference solution

Uncertainty component	Quantity	Uncertainty	Relative uncertainty
V_{Stock}	5 mL	0.0165 mL	0.00331
V_{Final}	10 mL	0.0172 mL	0.00172

The concentration of the fifth standard solution is calculated as:

$$C_{ref5} = \frac{5\ mL \cdot 1.000\ mg/mL}{50\ mL} = 0.500\ mg/mL$$

The uncertainty of the concentration of the fifth reference solution is:

$$\frac{u(C_{ref5})}{C_{ref5}} = \sqrt{(0.00331)^2 + (0.00172)^2} = 0.00373$$

$$u(C_{ref5}) = 0.00373 \ \cdot 0.500 = 0.001863\, mg/mL$$

2.3.3 Component 3. Uncertainty of the slope and intercept of the calibration curve

There are four main sources of uncertainty to consider when estimating uncertainty of the estimated concentration x_{pred}.

1. Random variations in absorbance (both reference y_i and measured y_{obs}).
2. Random effects in assigned reference value, x_i.
3. Constant unknown offset on x_i and y_i.
4. The assumption of linearity may not be valid [1].

However, the most significant of these sources is the first source, i.e., random variations in measurement of y_i and y_{obs}, which are estimated from the residual standard deviation S [1, 4].

$$S(residual) = \sqrt{\frac{\sum_{j=1}^{n}[Ai - (a + b \cdot c_i)]^2}{n-2}}$$

$$S(residual) = \sqrt{\frac{\sum_{i=0}^{n}[y_i - y_c]^2}{n-2}}$$

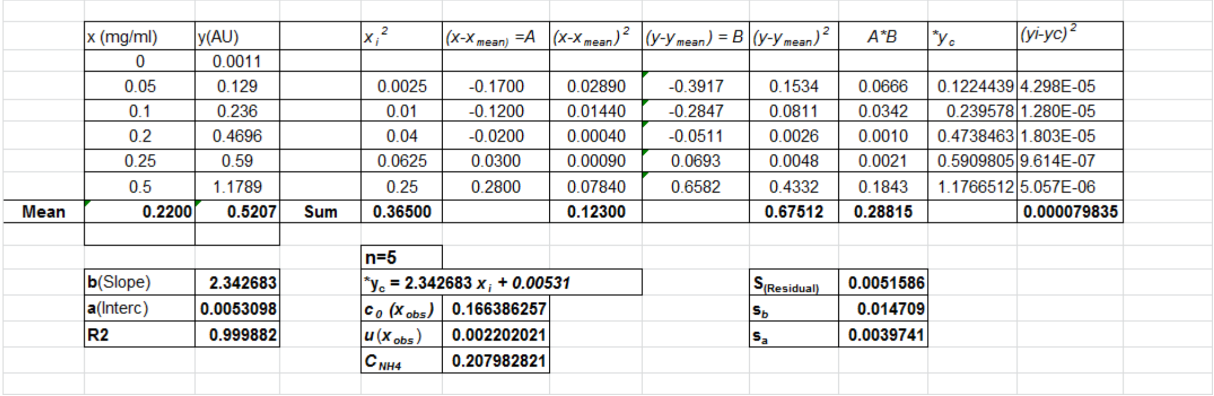
The uncertainty in x_{obs} (the determined concentration) is estimated as:

$$u(x_{obs}) = \frac{S(residual)}{b}$$

Table 10. Concentration levels of reference standards and their responses (AU)

	Concentration, x_i (mg/L)	Absorbance, y_i (AU)
Blank	0	0.0010
Ref Std 1	0.05	0.1290
Ref Std 2	0.10	0.2360
Ref Std 3	0.20	0.4696
Ref Std 4	0.25	0.5900
Ref Std 5	0.50	1.1789

Table 11. Calibration results



	x (mg/ml)	y(AU)		x_i^2	$(x\text{-}x_{mean})=A$	$(x\text{-}x_{mean})^2$	$(y\text{-}y_{mean}) = B$	$(y\text{-}y_{mean})^2$	A*B	$^*y_c$	$(yi\text{-}yc)^2$
	0	0.0011									
	0.05	0.129		0.0025	-0.1700	0.02890	-0.3917	0.1534	0.0666	0.1224439	4.298E-05
	0.1	0.236		0.01	-0.1200	0.01440	-0.2847	0.0811	0.0342	0.239578	1.280E-05
	0.2	0.4696		0.04	-0.0200	0.00040	-0.0511	0.0026	0.0010	0.4738463	1.803E-05
	0.25	0.59		0.0625	0.0300	0.00090	0.0693	0.0048	0.0021	0.5909805	9.614E-07
	0.5	1.1789		0.25	0.2800	0.07840	0.6582	0.4332	0.1843	1.1766512	5.057E-06
Mean	**0.2200**	**0.5207**	**Sum**	**0.36500**		**0.12300**		**0.67512**	**0.28815**		**0.000079835**
				n=5							
	b(Slope)	**2.342683**		$^*y_c$ **= 2.342683** x_i **+ *0.00531***				$S_{(Residual)}$	**0.0051586**		
	a(Interc)	**0.0053098**		$c_0\ (x_{obs})$	**0.166386257**			s_b	**0.014709**		
	R2	**0.999882**		$u(x_{obs})$	**0.002202021**			s_a	**0.0039741**		
				C_{NH4}	**0.207982821**						

Therefore, $y = 0.00531 + 2.34268x$ and

$c_0\ (x_{obs})$ = (0.3951 – 0.00531) / 2.34268 = 0.166386 mg/ml

Using this equation y_c can be calculated with known values for x and their corresponding square of difference, i.e., $[y_i - y_c]^2$.

$$S(residual) = \sqrt{\frac{\sum_{i=0}^{n}[y_i - y_c]^2}{n-2}} = \sqrt{\frac{9.0965 \cdot 10^{-5}}{3}} = 0.0051586\ \mathrm{AU}$$

$$u(x_{obs}) = \frac{S(residual)}{b} = \frac{0.0051586}{2.342683} = 0.002220\ \mathrm{mg/L}$$

2.3.4 Component 4: Uncertainty of the absorbance of the sample solution

Uncertainty of absorbance $u(A_{NH4})$

Table 12. Values of the uncertainties related to $u(A_{sample})$

Description	Value	Unit
$u(A_{NH4} repeatability)$	0.0012	AU
$u(A_{NH4} drift)$	0.0014	AU
$u(A_{NH4} interfereng)$	0.0025	AU

$$u(A_{sample}) = \sqrt{u(A_{NH4} repeatability)^2 + u(A_{NH4} drift)^2 + u(A_{NH4} interfereng)^2}$$

$$u(A_{NH4}) = \sqrt{0.0012^2 + 0.0014^2 + 0.0025^2} = 0.00311\ \mathrm{AU}$$

Table 13. Values of the uncertainties related to absorbance of the sample solution

Description	Value	Unit
$u(A_{NH4})$	0.00311	AU
$u(\Delta C_{dc})$	0.002*	mg/mL

*The uncertainty of ΔC_{dc} is established based on experience to 0.002 mg/mL.

2.4 Step 4. Calculation of combined standard uncertainty and expanded uncertainty

Table 14. Calculated values for uncertainties

	Description	Value	Standard uncertainty *u(x)*	Relative standard uncertainty *u(x)/x*
$\mathrm{C}_{\mathrm{Stock}}$	Stock solution concentration	1.000 mg/L	-	-
C_{Ref1}	Reference standard concentration	0.050 mg/L	0.000169 mg/L	0.0033845
C_{Ref2}	Reference standard concentration	0.100 mg/L	0.000342 mg/L	0.0035501
C_{Ref3}	Reference standard concentration	0.020 mg/L	0.000683 mg/L	0.0034157
C_{Ref4}	Reference standard concentration	0.250 mg/L	0.000867 mg/L	0.0034664
C_{Ref5}	Reference standard concentration	0.500 mg/L	0.001863 mg/mL	0.0037266
x_{Obs}	Observed concentration (c_0)	0.166 mg/mL	0.0022020 mg/mL	0.0132344
A_{sample}	Absorbance of sample	0.3951 AU	0.003106 AU	0.007862
fd	Dilution factor	1.25	0.001677	0.001341

Combined standard uncertainty is calculated as:

$$\frac{u_c(C_{\mathrm{NH}_4^+})}{(C_{\mathrm{NH}_4^+})} = \sqrt{(\frac{u(C_{ref1})}{C_{ref1}})^2 + \frac{u(C_{ref2})}{C_{ref2}})^2 + \frac{u(C_{ref3})}{C_{ref3}})^2 + \frac{u(C_{ref4})}{C_{ref4}})^2 + \frac{u(C_{ref5})}{C_{ref5}})^2 + \frac{u(x_{obs})}{x_{obs}})^2 + (\frac{u(A_{NH4})}{A_{NH4}})^2 + (\frac{u(fd)}{fd})^2}$$

$$\frac{u_c(C_{\mathrm{NH}_4^+})}{(C_{\mathrm{NH}_4^+})} = \sqrt{(0.0033845)^2 + (0.0035501)^2 + (0.0034157)^2 + (0.0034664)^2 + (0.0037266)^2 + (0.0132344)^2 + (0.007862)^2} + (0.001341)^2 = 0.017305$$

Calculation of the ammonium concentration

The absorbance of a sample solution containing the analyte and impurities, is used for measurement of the concentration. The ammonium concentration is calculated by using the following equation:

The output quantity ($C_{\mathrm{NH}_4^+}$) is calculated by means of the following function [2]:

$(C_{\mathrm{NH}_4^+}) = c_0 \cdot fd + C_{dc}$, Where: $c_0 = \left[\frac{A_{sample} - a}{b}\right]$

Table 15. Values for calculation of the $C_{\mathrm{NH}_4^+}$

Quantity	**Value**	**Unit**
$\mathrm{A}_{\mathrm{Sample}}$	0.3951	AU
a (Intercept)	0.00531	AU
b (Slope)	2.342683	AU x L/mg
R	0.9999	-
fd	1.25	-
ΔC_{dc}	0.000	mg/L
c_0=0.1664 mg/L and $C_{\mathrm{NH}_4^+}$ in the sample **=** 0.208 mg/L		

$$u_c\left(C_{\mathrm{NH}_4^+}\right) = 0.0173 \cdot 0.2084 = 0.00360 \text{ mg/L}$$

$$u_c\left(C_{\mathrm{NH}_4^+}\right) = \sqrt{u_c\left(C_{\mathrm{NH}_4^+}\right)^2 + u_c\left(C_{dc}\right)^2} = 0.00412 \text{ mg/L}$$

Expanded uncertainty (k = 2, approximately 95% level of confidence) is:

$U = 2 \cdot u_c = 0.0082\, mg/L$

2.5 Reporting of result

The result is expressed as:

Content of NH_4^+ = 0.208 mg/L ± $0.008\, mg/L$ (*k* = 2, approximately 95% level of confidence)

Approach 3

The purpose is to estimate uncertainty for the determined ammonium ion concentration in water by taking into account the uncertainties in the reference standard solutions and the determined NH_4^+ concentration ($u(c_0)$ or $u(x_{obs})$ [1].

2.1 Step 1. Specification of a measurand

The output quantity ($C_{\mathrm{NH}_4^+}$) is calculated by means of the following function [2]:

$$(C_{\mathrm{NH}_4^+}) = \left[\frac{A_{sample} - a}{b}\right] \cdot fd + C_{dc}$$

Where:
$c_0 = \left[\frac{A_{sample} - a}{b}\right]$

Where:
A_{sample}: measured absorbance of the sample solution;
a and b: intercept and slope of the calibration curve ($y = a + bx$)
C_{dc}: component taking into account impurities

2.2 Step 2: Identification of uncertainty sources

Table 1. Uncertainty components

Input variable	Uncertainty components	$u(x_i)$
A_{sample}	Interferences Repeatability Drift of photometer	$u(A_{sample})$
a and *b*	Purity of standard material Preparation of solutions Repeatability and drift of photometer	$u(a)$ $u(b)$
f_d	Preparation of sample solution	$u(fd)$
C_{dc}	Contamination Decomposition Volatilization Incomplete reaction	$u(C_{dc})$

Each of the parameters that affect the value of the measurand are shown as a cause and effect diagram (Fig. 1) [1,2].

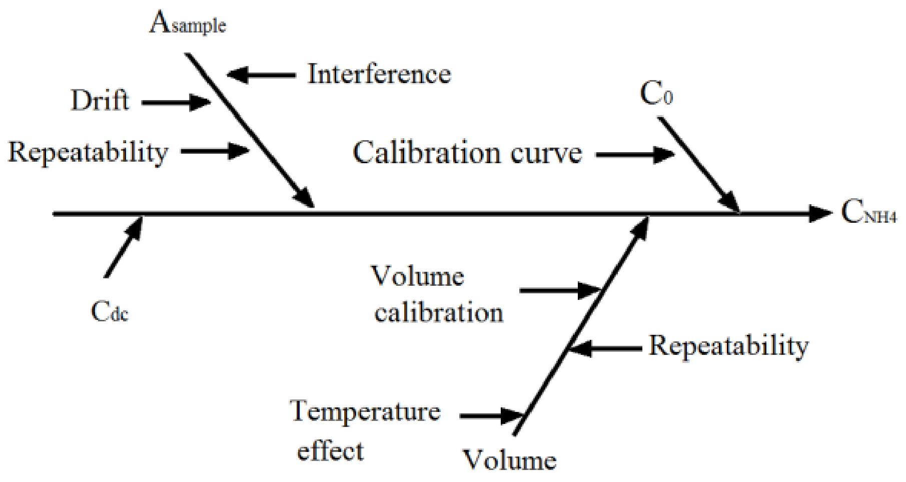


Fig. 1 Cause and effect diagram for determination of $C_{\mathrm{NH}_4^+}$

2.3 Step 3. Quantification of uncertainty components

2.3.1 Component 1: Uncertainty of preparation of the sample

Uncertainty of preparation of the sample is associated with the uncertainty of the dilution factor, calculated as:

$$fd = \frac{V_2}{V_1} = \frac{25mL}{20\,mL} = 1.25$$

The standard uncertainty of the dilution factor $u(fd)$ is:

$$\frac{u(fd)}{fd} = \sqrt{(\frac{u(V_1)}{V_1})^2 + (\frac{u(V_2)}{V_2})^2}$$

The standard uncertainties $u(V_i)$ are calculated as:

$$u(V_i) = \sqrt{u(Vcal)^2 + u(Vrep)^2 + u(VT)^2}$$

Table 2. Uncertainty values of the dilution volume (calculation of these values is explained in Annex 1.1)

mL	Volume	$u(VT)$	$u(Vrep)$	$u(Vcal)$	$u(V_i)$	$\frac{u(V_i)}{V_i}$
V_1	20 mL	0.0097 mL	0.0083 mL	0.01633 mL	0.0207 mL	0.00104
V_2	25 mL	0.01212 mL	0.0125 mL	0.01225 mL	0.0213 mL	0.00085

$$\frac{u(fd)}{fd} = \sqrt{(0.00104)^2 + (0.00085)^2} = 0.00134$$

$$u(fd) = 0.00166 \cdot 1.25 = 0.001677$$

2.3.2 Component 2: Uncertainty of the absorbance of the sample solution

Uncertainty of absorbance $u\left(A_{sample}\right)$

Table 3. Values of the uncertainties related to $u\left(A_{sample}\right)$

Description	Value	Unit
$u\left(A_{sample}repeatability\right)$	0.0012	AU
$u\left(A_{sample}drift\right)$	0.0014	AU
$u\left(A_{sample}interfereng\right)$	0.0025	AU

$$u\left(A_{sample}\right) = \sqrt{u\left(A_{sample}repeatability\right)^2 + u\left(A_{sample}drift\right)^2 + u\left(A_{sample}interfereng\right)^2}$$

$$u\left(A_{sample}\right) = \sqrt{0.0012^2 + 0.0014^2 + 0.0025^2} = 0.00311\text{ AU}$$

Table 4. Values of the uncertainties related to absorbance of the sample solution

Description	Value	Unit
$u\left(A_{sample}\right)$	0.00311	AU
$u(C_{dc})$	0.002*	mg/mL

*The uncertainty of C_{dc} is established based on experience to 0.002 mg/mL.

2.3.3 Component 3 Calibration curve

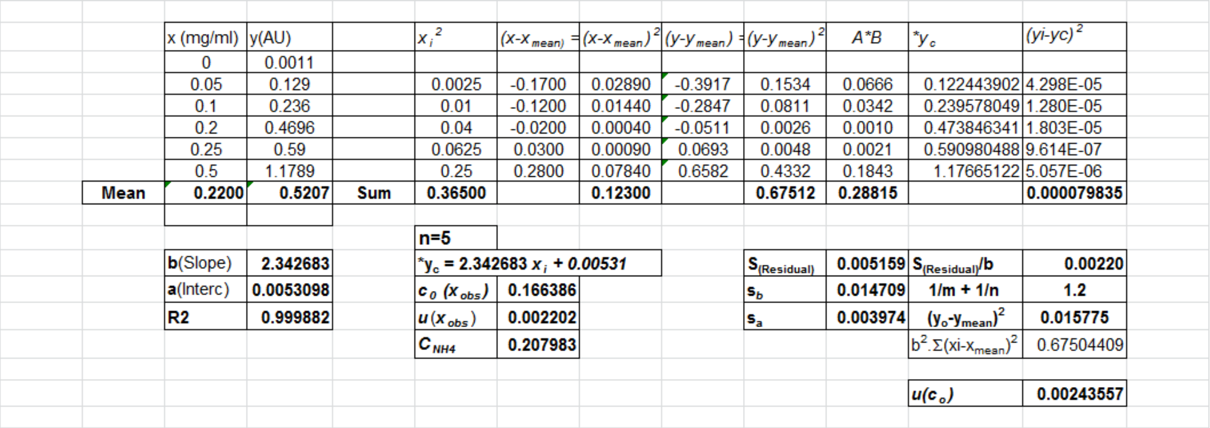
Estimation of the uncertainty in ($u(c_0)$ or $u(x_{obs})$:

$$u(c_0) = \frac{S_{residual}}{b} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum(x_i - \bar{x})^2}}$$

(it has units of mg/L)

b	Slope
y-bar	average value of the observed responses (= $\Sigma y_i/n$)
y_0	response of the sample
x_i	concentration of the i:th standard solution
x-bar	average value of the concentration of the standard solutions (= $\Sigma x_i/n$)
m	number of measurements to determine C_{NH4}
n	number of calibration points

Table 5. Calibration results



	x (mg/ml)	y(AU)		x_i^2	$(x-x_{mean})$	$(x-x_{mean})^2$	$(y-y_{mean})$	$(y-y_{mean})^2$	*A*B*	$*y_c$	$(yi-yc)^2$
	0	0.0011									
	0.05	0.129		0.0025	-0.1700	0.02890	-0.3917	0.1534	0.0666	0.122443902	4.298E-05
	0.1	0.236		0.01	-0.1200	0.01440	-0.2847	0.0811	0.0342	0.239578049	1.280E-05
	0.2	0.4696		0.04	-0.0200	0.00040	-0.0511	0.0026	0.0010	0.473846341	1.803E-05
	0.25	0.59		0.0625	0.0300	0.00090	0.0693	0.0048	0.0021	0.590980488	9.614E-07
	0.5	1.1789		0.25	0.2800	0.07840	0.6582	0.4332	0.1843	1.17665122	5.057E-06
Mean	**0.2200**	**0.5207**	**Sum**	**0.36500**		**0.12300**		**0.67512**	**0.28815**		**0.000079835**
				n=5							
	b(Slope)	**2.342683**		**$*y_c$ = 2.342683 x_i + *0.00531***				**$S_{(Residual)}$**	**0.005159**	**$S_{(Residual)}$/b**	**0.00220**
	a(Interc)	**0.0053098**		***c_0 (x_{obs})***	**0.166386**			**s_b**	**0.014709**	**1/m + 1/n**	**1.2**
	R2	**0.999882**		***u*(x_{obs})**	**0.002202**			**s_a**	**0.003974**	**$(y_o-y_{mean})^2$**	**0.015775**
				C_{NH4}	**0.207983**					$b^2.\Sigma(xi-x_{mean})^2$	0.67504409
										$u(c_o)$	**0.00243557**

$$S(residual) = \sqrt{\frac{\sum_{i=0}^{n}[y_i - y_c]^2}{n-2}} = \sqrt{\frac{7.9835 \cdot 10^{-5}}{3}} = 0.005159 \text{ AU}$$

$$u(c_0) = \frac{S_{residual}}{b} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum (x_i - \bar{x})^2}} = \frac{0.005159}{2.3468} \cdot \sqrt{\frac{1}{1} + \frac{1}{5} + \frac{0.015775}{0.67504}} = 0.002436\, mg/L$$

2.4 Step 4. Calculation of Combined standard uncertainty and Expanded Uncertainty

2.4.1 Calculation of Combined standard uncertainty

$$\frac{u\ (c_{NH4})}{(c_{NH4})} = \sqrt{\left(\frac{u(A_{sample})}{A_{sample}}\right)^2 + \left(\frac{u(c_0)}{c_0}\right)^2 + \left(\frac{u(fd)}{fd}\right)^2}$$

$$\frac{u(c_{NH4})}{(c_{NH4})} = \sqrt{\left(\frac{0.00311}{0.3951}\right)^2 + \left(\frac{0.002202}{0.16639}\right)^2 + \left(\frac{0.00168}{1.25}\right)^2} = 0.01545$$

2.4.2 *Calculation of the output quantity*

Table 16. Values for calculation of the $C_{\mathrm{NH_4^+}}$

Quantity	**Value**	**Unit**
A_{Sample}	0.3951	AU
a (Intercept)	0.00531	AU
b (Slope)	2.34268	AU x L/mg
R	0.9999	-
fd	1.25	-
ΔC_{dc}	0.000	mg/L
c_0=0.1664 mg/L and $C_{\mathrm{NH_4^+}}$ in the sample **=** 0.2079 mg/L		

$$u\left(C_{\mathrm{NH_4^+}}\right) = 0.01545 \cdot 0.2079 = 0.0032125 \text{ mg/L}$$

Combined standard uncertainty is:

$$u_c\left(C_{\mathrm{NH_4^+}}\right) = \sqrt{u_c\left(C_{\mathrm{NH_4^+}}\right)^2 + u_c\left(C_{dc}\right)^2} = 0.00378\ \mathrm{mg/L}$$

2.4.3 Expanded uncertainty is:

$$U = k \cdot u_c\left(C_{\mathrm{NH_4^+}}\right) = 2 \cdot 0.0038\ \mathrm{mg/L}$$

$U = 0.0076\ \mathrm{mg/L}$ (k =2, approximately 95% level of confidence)

2.5 Reporting of results

$C_{\mathrm{NH_4^+}}$ = 0.208 ± 0.008 mg/L (k = 2, approximately 95% level of confidence).

3. Estimation of the combined uncertainty using spreadsheet

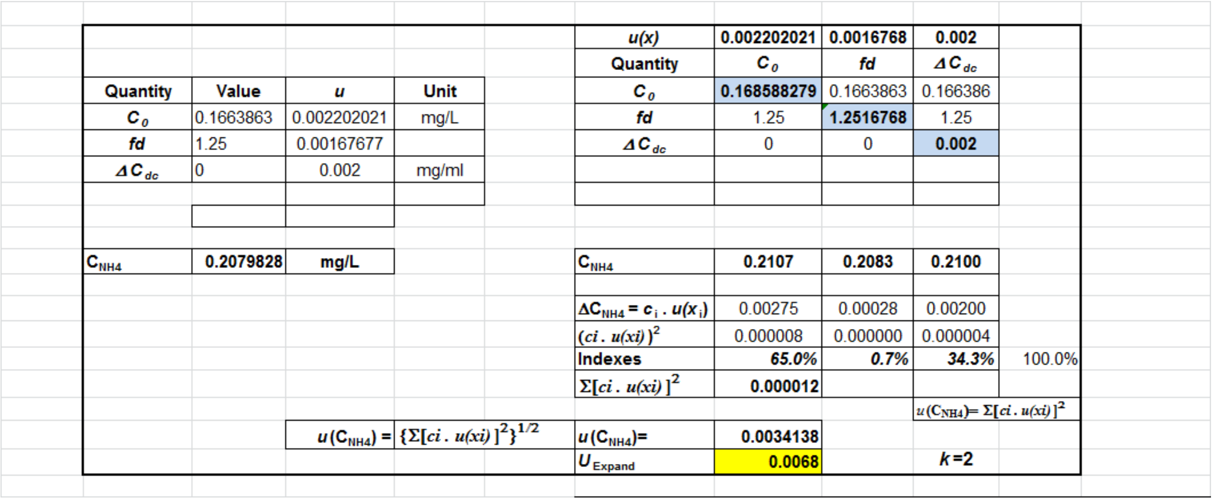
The uncertainty of the final concentration of $\mathrm{NH_4^+}$ can also be estimated by applying the spreadsheet approach. This approach summarizes the relationship between all of the variables that have impact on the uncertainty of the concentration of $\mathrm{NH_4^+}$.

The following equation is used for the calculation of the combined uncertainty:

$$u_c = \sqrt{\left[\left(\frac{\delta C_{sample}}{\delta Co}\right)^2 \cdot u^2(co) + \left(\frac{\delta C_{sample}}{\delta fd}\right)^2 \cdot u^2(fd) + \left(\frac{\delta C_{sample}}{\delta \Delta Cdc}\right)^2 \cdot u^2(\Delta Cdc)\right]}$$

The equation neglects any correlation between the measured values.

Table 7 Estimation of the combined uncertainty by the spread sheet approach



Quantity	Value	u	Unit
C_0	0.1663863	0.002202021	mg/L
fd	1.25	0.00167677	
ΔC_{dc}	0	0.002	mg/ml

C_{NH4}	**0.2079828**	**mg/L**

$u(C_{NH4}) = \{\Sigma[ci\ .\ u(xi)]^2\}^{1/2}$

$u(x)$	**0.002202021**	**0.0016768**	**0.002**	
Quantity	C_0	fd	ΔC_{dc}	
C_0	**0.168588279**	0.1663863	0.166386	
fd	1.25	**1.2516768**	1.25	
ΔC_{dc}	0	0	**0.002**	
C_{NH4}	**0.2107**	**0.2083**	**0.2100**	
$\Delta C_{NH4} = c_i\ .\ u(x_i)$	0.00275	0.00028	0.00200	
$(ci\ .\ u(xi))^2$	0.000008	0.000000	0.000004	
Indexes	***65.0%***	***0.7%***	***34.3%***	100.0%
$\Sigma[ci\ .\ u(xi)]^2$	**0.000012**			
			$u(C_{NH4}) = \Sigma[ci\ .\ u(xi)]^2$	
$u(C_{NH4})=$	**0.0034138**			
U_{Expand}	**0.0068**		k=2	

$$u_c\left(C_{NH_4}\right) = \sqrt{\sum\left(\Delta C_{NH_4}\right)^2} = 0.0034\ \mathrm{mg/L}$$

Calculation of the output quantity

$$C_{\mathrm{NH_4^+}} = 0.2079\ \mathrm{mg/L}$$

Hence, $C_{\mathrm{NH_4^+}} = 0.208 \pm 0.007\ \mathrm{mg/L}\ (\mathrm{k}\ = 2, \text{approximately 95\% level of confidence})$

4. Summary

The results of uncertainty estimations, using different methods, are summarised in the following Table.

Table 8 Summary of the obtained results for $u_c(C_{NH_4})$ using different approaches

Estimation of $u_c(C_{NH_4})$	Determined concentration (mg/L)	Estimated U_{Expanded} (k = 2) mg/mL
Approach 1	0.2084	0.007
Approach 2	0.2084	0.008
Approach 3	0.2084	0.007

5. References

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