European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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General European OMCL Network (GEON)
QUALITY MANAGEMENT DOCUMENT

PA/PH/OMCL (18) 148 R2 CORR

EVALUATION OF MEASUREMENT UNCERTAINTY
ANNEX 1.3

Full document title and reference	Evaluation of Measurement Uncertainty ANNEX 1: ESTIMATION OF MEASUREMENT UNCERTAINTY OF USING BOTTOM-UP APPROACH Annex 1.3: Estimation of Measurement Uncertainty for Assay using HPLC Method *PA/PH/OMCL (18) 148 R2 CORR*
Document type	Guideline
Legislative basis	Council Directive 2001/83/EC and 2001/82/EC, as amended
Date of first adoption	19 July 2019
Date of original entry into force	1 February 2020
Date of entry into force of revised document	
Previous titles/other references / last valid version	
Custodian Organisation	The present document was elaborated by the OMCL Network / EDQM of the Council of Europe
Concerned Network	GEON

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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 1.3 Estimation of the measurement uncertainty for assay using HPLC method

Example: Determination of content of active ingredient in rosuvastatin tablets using HPLC method

1. Description of the analytical procedure:

Preparation of standard solution:

21.0 mg (accurate mass) of rosuvastatin calcium working standard is diluted to 100.0 mL with the solvent.

Preparation of sample solution:

Weigh 20 tablets, calculate the average mass (n=20) and pulverize the tablets. Accurately weigh a quantity of pulverized tablets corresponding to one average mass and transfer it to a 50.0 mL volumetric flask, add around 25 mL solvent and dissolve using ultrasonic bath for approximately 20 minutes. Allow to cool to room temperature and complete to volume with the solvent. Filter using syringe filter 0.45µm.

Procedure: 10 µL of standard and sample solution are injected into the HPLC system.

The content of rosuvastatin is expressed as per cent of the declared content (10 mg rosuvastatin/tablet).

Limits: 95.0% - 105.0% of the declared content

2. Estimation of the measurement uncertainty

2.1 Step 1. Specification of a measurand

The measurand is the content of rosuvastatin in tablets, expressed as per cent of the declared content, calculated by following formula:

$$\text{Rosuvastatin }\%_{of\ the\ decl.\ content} = \frac{A_{sample} \cdot m_{st} \cdot P_{st} \cdot 50}{A_{st} \cdot m_{sample} \cdot 100} \cdot \frac{2 \cdot M_{Rosuvastatin}}{M_{RosuvastatinCa}} \cdot m_{average} \cdot \frac{100}{10}$$

Where:

A_{sample}: peak area of rosuvastatin in the chromatogram obtained with sample solution

A_{st}: peak area of rosuvastatin in the chromatogram obtained with standard solution

m_{st}: mass (mg) of the working standard, used for preparation of the standard solution

P_{st}: purity (%) of the rosuvastatin calcium working standard

m_{sample}: mass (mg) of the pulverized tablets used for preparation of the sample solution

$m_{average}$: average mass (mg) (n=20)

$M_{Rosuvastatin}$: molar mass (483.54 g/mol) of rosuvastatin ($C_{22}H_{28}FN_3O_6S$)

$M_{RosuvastatinCa}$: molar mass (1001.14 g/mol) of rosuvastatin calcium ($C_{44}H_{54}CaF_2N_6O_{12}S_2$)

100, 50: dilution volumes (mL) of standard solution and sample solution, respectively

10: declared content (mg)

100: conversion in per cent

2.2 Step 2. Identification of uncertainty contributors

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

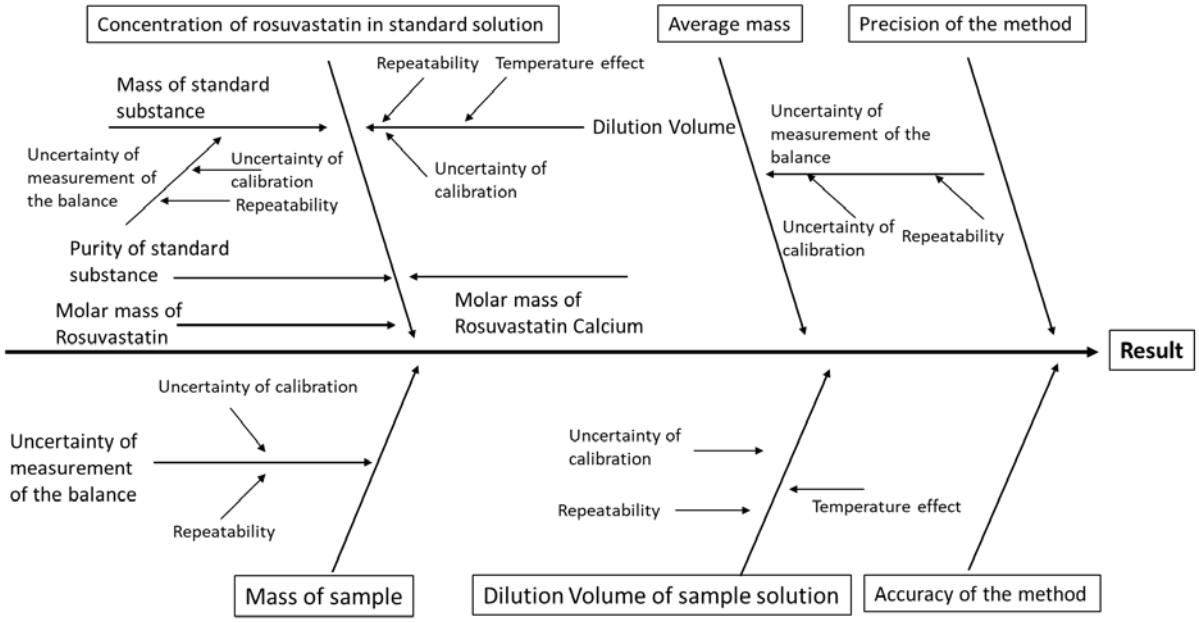

Fig. 1 Cause and effect diagram for HPLC determination of content of Rosuvastatin

2.3 Step 3. Quantification of uncertainty components.

2.3.1 Component 1: Concentration of Rosuvastatin in standard solution

2.3.1.1 *Uncertainty of the mass of Rosuvastatin calcium working standard*

Uncertainty of usage of the balance

The stated uncertainty of measurement of the weighing result from the certificate of calibration (uncertainty of usage of the balance, k = 2, approximately 95 % level of confidence) is:

$$\mathrm{U(W)} = (4.08 \cdot 10^{-5}\mathrm{g} + 1.53 \cdot 10^{-5}R)$$

The uncertainty of the mass of the standard substance (R = 21.0 mg) is:

$$\mathrm{U(W)} = (4.08 \cdot 10^{-5}\mathrm{g} + 1.53 \cdot 10^{-5} \cdot 0.0210\mathrm{g}) = 0.0411\ \mathrm{mg}$$

Standard uncertainty of the measurement of the mass of the standard substance is:

$$u(m_{st}) = \frac{\mathrm{U(W)}}{2} = 0.02055\ \mathrm{mg}$$

The relative standard uncertainty of measurement of the mass of the standard substance is:

$$\frac{u(m_{st})}{(m_{st})} = \frac{0.02055\ mg}{21.0\ mg} = 0.000979$$

2.3.1.2 *Purity of Rosuvastatin calcium working standard*

The supplier quotes the purity of rosuvastatin calcium working standard in the certificate of analysis as 100.69% ± 0.01% without information on level of confidence and distribution.
In case of purity a rectangular distribution is assumed [1], so the uncertainty of the purity was divided by square root of 3 to obtain the standard uncertainty $u(\mathrm{Pst})$ *as*:

$$u(\mathrm{Pst}) = \frac{0.01}{\sqrt{3}} = 0.00577\ \%$$

The relative standard uncertainty of measurement of the purity is:

$$\frac{u(\mathrm{Pst})}{(\mathrm{Pst})} = \frac{0.00577\ \%}{100.69\ \%} = 0.0000573$$

Calculation of standard Uncertainty of mass of Rosuvastatin calcium working standard:

Table 1 provides summary of calculated values of uncertainties of the mass of rosuvastatin calcium working standard.

Table 1 Summary of values of uncertainties of the mass of rosuvastatin calcium working standard

Description	**Value x**	*u(x)*	*u(x)/x*
Purity	100.69 %	0.00577 %	0.0000573
Mass	21.0 mg	0.02055 mg	0.000979

$$\frac{u(m_{st})}{m_{st}} = \sqrt{(0.0000573)^2 + (0.000979)^2} = 0.00098$$

The uncertainty must be calculated in the same unit, i.e. mg, as the result.

$$u(m_{st}) = 0.00098 \cdot 21.0\ \mathrm{mg} = 0.0206\ \mathrm{mg}$$

2.3.1.3 *Uncertainty of the dilution volume of the standard solution*

Volume Calibration

According to the manufacturer certificate, the uncertainty of measurement of the volumetric flask of 100.0 mL is ± 0.1 mL, at a temperature of 20 °C, without any level of confidence and any distribution information. A triangular distribution is assumed, because the actual volume is more likely to be at the center than at the extremes of the range [1]. Therefore the uncertainty in calibration of volume is:

$$u(Vstcal) = \frac{0.1}{\sqrt{6}} = 0.04\ mL$$

Repeatability of the Volume Measurements (run-to-run variation)

A series of ten fill-and-weigh exercises on the volumetric flask gave a standard deviation $u(VstRep)$ of 0.07815 mL, being used in the final calculation directly.

$u(VstRep) =$ 0.07815 mL

Temperature Effect

The volumetric flask has been calibrated at 20 ºC whereas the laboratory temperature varies between 16 ºC and 24 ºC (20 ºC ± 4 ºC). The uncertainty from this effect is calculated from the estimate of temperature range and the coefficient of volume expansion, i.e. 0.00021 per 1 ºC [1, 2].

$$\text{Volume expansion } = 100 \text{ mL} \cdot 4\ ^{\circ}\text{C} \cdot \frac{0.00021}{1\ ^{\circ}\text{C}} = 0.084 \text{ mL}$$

Assuming rectangular distribution, the uncertainty for temperature variations is:

$$u(VstT) = \frac{0.084}{\sqrt{3}} = 0.048\ mL$$

Calculation of the Standard Uncertainty of dilution volume of the standard solution *u(Vst).*

$$u(Vst) = \sqrt{u(Vstcal)^2 + u(VstRep)^2 + u(VstT)^2}$$

$$u(Vst) = \sqrt{0.04^2 + 0.07815^2 + 0.048^2} = 0.100 \text{ mL}$$

The relative standard uncertainty of dilution volume of the standard solution is:

$$\frac{u(Vst)}{(\text{Vst})} = \frac{0.100 \text{ mL}}{100.0 \text{ mL}} = 0.00100$$

2.3.1.4 *Uncertainty of the molar mass determination*

Standard atomic weights and quoted uncertainties for the constituent elements of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) and rosuvastatin calcium ($C_{44}H_{54}CaF_2N_6O_{12}S_2$) are provided in Table 2 [5].

Table 2**.** Standard atomic weights and quoted uncertainties for the constituent elements of rosuvastatin ($C_{22}H_{28}FN_3O_6S$) and rosuvastatin calcium ($C_{44}H_{54}CaF_2N_6O_{12}S_2$)

Element	**Standard Atomic Weight**	**Quoted Uncertainty** *u(e)*	**Standard Uncertainty** $u(e)/\sqrt{3}$
Carbon C	12.0106	± 0.001	0.0006
Oxygen O	15.9994	± 0.00037	0.00021
Hydrogen H	1.007975	± 0.000135	0.000078
Nitrogen N	14.00674	± 0.00042	0.000245
Sulphur S	32.0675	± 0.0085	0.0049
Fluorine F	18.998403	± 0.006	0.00346
Calcium Ca	40.078	± 0.004	0.0023

The molar mass of rosuvastatin is:

$$M_{Rosuvastatin} = 22 \cdot 12.0106 + 28 \cdot 1.007975 + 18.998403 + 3 \cdot 14.00674 + 6 \cdot 15.9994 + 32.0675 = 481.539 \text{ g/mol}$$

The standard uncertainty of molar mass of rosuvastatin is:

$$u(M_{Rosuvastatin}) = \sqrt{22 \cdot 0.0006^2 + 28 \cdot 0.000078^2 + 0.0346^2 + 3 \cdot 0.000245^2 + 6 \cdot 0.00021^2 + 0.0049^2} = 0.03507 \text{ g/mol}$$

The relative standard uncertainty of molar mass of rosuvastatin is:

$$\frac{u(M_{Rosuvastatin})}{M_{Rosuvastatin}} = \frac{0.03507 \text{ g/mol}}{481.539 \text{ g/mol}} = 0.000072829$$

The molar mass of rosuvastatin calcium is:

$$M_{RosuvastatinCa} = 2 \cdot (22 \cdot 12.0106 + 28 \cdot 1.007975 + 18.998403 + 3 \cdot 14.00674 + 6 \cdot 15.9994 + 32.0675) + 40.078 = 1003.156 \text{ g/mol}$$

The standard uncertainty of molar mass of rosuvastatin calcium is:

$$uM_{RosuvastatinCa} == \sqrt{44 \cdot 0.0006^2 + 56 \cdot 0.000078^2 + 2 \cdot 0.0346^2 + 6 \cdot 0.000245^2 + 12 \cdot 0.00021^2 + 2 \cdot 0.0049^2 + 0.0023^2} = 0.049645 \text{ g/mol}$$

The relative standard uncertainty of molar mass of rosuvastatin calcium is:

$$\frac{u(M_{RosuvastatinCa})}{M_{RosuvastatinCa}} = \frac{0.049645 \text{ g/mol}}{1003.156 \text{ g/mol}} = 0.0000495$$

Calculation of standard uncertainty of concentration of Rosuvastatin in standard solution

Table 3 provides summary of calculated values of uncertainty sources for preparation of standard solution.

Table 3 Summary of values of uncertainties of the uncertainty sources for preparation of standard solution

Description	**Value x**	***u(x)***	***u(x)/x***
Weighing standard substance	21.0 mg	0.0206 mg	0.00098
Dilution volume	100.0 mL	0.1000 mL	0.00100
$M_{Rosuvastatin}$	481.539 g/mol	0.03507 g/mol	0.0000728
$M_{RosuvastatinCa}$	1003.156 g/mol	0.049645 g/mol	0.0000495

Considering uncertainties in weighing and volume preparation the standard uncertainty of concentration of rosuvastatin in standard solution is:

$$\frac{u(Cst)}{C_{st}} = \sqrt{(0.00098)^2 + (0.00100)^2 + (0.0000728)^2 + (0.0000495)^2} = 0.001403$$

The concentration of rosuvastatin in standard solution is calculated as:

$$C_{st} = \frac{m_{rosuvastatin}}{V_{st}} = \frac{m_{st} \cdot P_{st}}{100} \cdot \frac{2 \cdot M_{Rosuvastatin}}{M_{RosuvastatinCa}} = \frac{21.0 \text{ mg} \cdot 1.0069}{100 \text{ mL}} \cdot \frac{963.08}{1001.14} = 0.2034 \text{ mg/mL}$$

The uncertainty of the concentration of rosuvastatin in the standard solution is:

$$u(Cst) = C_{st} \cdot 0.001403 = 0.2034 \cdot 0.001403 = 0.0002854$$

2.3.2 Component 2: Mass of sample

2.3.2.1 *Uncertainty of the mass of sample*

Uncertainty of usage of the balance

The stated uncertainty of measurement of the weighing result from the certificate of calibration (uncertainty of usage of the balance, k = 2, approximately 95 % level of confidence) is:

$$\mathrm{U(W)} = (4.08 \cdot 10^{-5}\mathrm{g} + 1.53 \cdot 10^{-5}R)$$

The uncertainty of the mass of the sample (R = 155.3 mg) is:

$$\mathrm{U(W)} = (4.08 \cdot 10^{-5}\mathrm{g} + 1.53 \cdot 10^{-5} \cdot 0.1553\ \mathrm{g}) = 0.00004317\ \mathrm{g} = 0.043\ \mathrm{mg}$$

Standard uncertainty of the measurement of the mass of the sample is:

$$u(m_{sample}) = \frac{\mathrm{U(W)}}{2} = 0.0216\ \mathrm{mg}$$

The relative standard uncertainty of measurement of the mass of the sample is:

$$\frac{u(m_{sample})}{(m_{sample})} = \frac{0.0216\ \mathrm{mg}}{155.3\ \mathrm{mg}} = 0.000139$$

Calculation of standard uncertainty of the mass of the sample:

Calculated values of uncertainty related to the mass of the sample are provided in Table 4.

Table 4 Summary of values of uncertainties of the mass of sample

Description	**Value x**	***u(x)***	***u(x)/x***
Mass of sample (mg)	155.3 mg	0.0236 mg	0.000139

2.3.3 Component 3: Dilution Volume of sample solution

2.3.3.1 *Uncertainty of the dilution volume of the sample solution*

Volume Calibration

According to the manufacturer certificate, the uncertainty of measurement of the volumetric flask of 50.0 mL is ± 0.06 mL, at a temperature of 20 °C, without any level of confidence and any distribution information. A triangular distribution is assumed, because the actual volume is more likely to be at the center than at the extremes of the range [1]. Therefore the uncertainty of the calibration of volume is:

$$u(Vsamplecal) = \frac{0.06}{\sqrt{6}} = 0.0245\ mL$$

Repeatability of the Volume Measurements (run-to-run variation)

A series of ten fill-and-weigh exercises on the volumetric flask gave a standard deviation *u(Vsample rep)* of 0.07082 mL, being used in the final calculation directly.

u(Vsample rep)$_=$ 0.07082 mL

Temperature Effect

The volumetric flask has been calibrated at 20 °C whereas the laboratory temperature varies between 16 °C and 24 °C (20 °C ± 4 °C). The uncertainty from this effect is calculated for the target volume (50 mL) from the estimate of temperature variation and the coefficient of volume expansion, i.e., 0.00021 per 1 °C [1, 2].

$$\text{Volume expansion } = 50\ \text{mL} \cdot 4\ ^{\circ}\text{C} \cdot \frac{0.00021}{1\ ^{\circ}\text{C}} = 0.042\ \text{mL}$$

Assuming rectangular distribution, the uncertainty for temperature variations is:

$$u(VsampleT) = \frac{0.042}{\sqrt{3}} = 0.024\ \text{mL}$$

Calculation of the Standard Uncertainty of the dilution volume of sample solution, $u(Vsample)$

$$u(Vsample) = \sqrt{u(Vsamplecal)^2 + u(Vsamplerep)^2 + u(VsampleT)^2}$$

$$u(Vsample) = \sqrt{0.0245^2 + 0.07082 + 0.024^2} = 0.07869\ \text{mL}$$

The relative standard uncertainty of measurement of the dilution volume of the sample solution is:

$$\frac{u(V_{sample})}{V_{sample}} = \frac{0.07869\ \text{mL}}{50\ \text{mL}} = 0.00157$$

2.3.4 Component 4: Uncertainty of Average mass

Weigh the mass of 20 tablets individually and calculate the average mass and standard deviation.

Calculated average mass / tablet: $m_{average}$ = 0.154895 g
Calculated standard deviation of the mass of 20 tablets $u_m = 0.000984$ mg

2.3.4.1 *Uncertainty of usage of the balance*

The stated uncertainty of measurement of the weighing result from the certificate of calibration (uncertainty of usage of the balance, k = 2, approximately 95% level of confidence) is:

$$\mathrm{U(W)} = (4.08 \cdot 10^{-5}\mathrm{g} + 1.53 \cdot 10^{-5} R)$$

The uncertainty of the weighing result (R = 154.9 mg) is:

$$\mathrm{U(W)} = (4.08 \cdot 10^{-5}\mathrm{g} + 1.53 \cdot 10^{-5} \cdot 0.154.9\ \mathrm{g}) = 0.0000432\ \mathrm{g} = 0.043\ \mathrm{mg}$$

Standard uncertainty of the measurement of the weighing result is:

$$u(weighing\ result) = \frac{\mathrm{U(W)}}{2} = 0.0216\ \mathrm{mg}$$

2.3.4.2 *Uncertainty related to Repeatability*

Considering triangular distribution [1)] in the manufacture of tablets, the uncertainty is:

$$u(brep) = \frac{u_m}{\sqrt{6}} = \frac{0.000984}{\sqrt{6}} = 0.0004\ mg$$

[1)] a triangular distribution is chosen because the production process of pharmaceutical formulations is a controlled, the mean value is more likely than extreme, and so the triangular distribution is reasonably accepted than the rectangular distribution.

Uncertainty of average mass:

$$u(m_{average}) = \sqrt{u(weighing\ result)^2 + u(brep)^2} = \sqrt{(0.0216)^2 + (0.0004)^2} = 0.0216\ \mathrm{mg}$$

$$\frac{u(m_{average})}{(m_{average})} = \frac{0.0216\ mg}{154.895\ mg} = 0.000139$$

2.3.5 Component 5: Precision of the method

The method validation shows a repeatability of determination $u(rep)$ = 1.01 % (0.0101). This value can be used directy for calculation of combined standard uncertainty of the final result.

2.3.6 Component 6: Accuracy of the method

The method validation shows a mean recovery values of R = 99.32% or 0.9932 and Relative standard deviation, RSD = 1.03 % (n = 9). The standard uncertainty is calculated as the standard deviation of the mean:

$$u_R = \frac{0.0103}{\sqrt{9}} = 0.0034$$

A Student t-test is used to determine whether the mean recovery is significantly different from 1. The test statistic t is calculated as:

$$t = \frac{|1 - R|}{u_R} = \frac{|1 - 0.9932|}{0.0034} = 2.0$$

This value is compared with the 2-tailed critical value t_{crit}, for n-1 degrees of freedom at approximately 95 % level of confidence (where n is the number of results used to estimate the recovery n = 9).

$$t = 2.0 < t_{critical}(2.3)$$

The mean recovery is considered as not significantly different from 1 and therefore the bias may be neglected [1].

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

Combined standard uncertainty is calculated as:

$$\frac{u_c}{\text{Rosuvastatin \%}_{of\ the\ decl.\ content}} = \sqrt{(\frac{u(Cst)}{C_{st}})^2 + (\frac{u(m_{sample})}{m_{sample}})^2 + (\frac{u(V_{sample})}{V_{sample}})^2 + (\frac{u(m_{average})}{(m_{average})})^2 + u(rep)^2}$$

$$\frac{u_c}{\text{Rosuvastatin \%}_{of\ the\ decl.\ content}} = \sqrt{(0.001403)^2 + (0.000139)^2 + (0.00157)^2 + (0.000139)^2 + (0.0101)^2} = 0.010319009$$

$$u_c = 0.010319009 \cdot 100.5 = 1.037060425\ \%$$

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

$U = 2 \cdot u_c = 2.07412085\ \%$

2.5 Reporting of result

The result is expressed as:

Content of Rosuvastatin = 100.5 % ± 2.1% (k=2, approximately 95% level of confidence)

3. References

1. Eurachem/CITAC Guide CG 4, Quantifying Uncertainty in Analytical Measurement, Third Edition, 2012.
2. A Guide on Measurement Uncertainty in Chemical & Microbiological Analysis. Accreditation Scheme for Laboratories, Technical Guide 2. Second edition 2008.
3. ISO 7870-2:2013, control charts, part 2: Shewhart control charts.
4. A Guide on Measurement Uncertainty in Chemical & Microbiological Analysis. Accreditation Scheme for Laboratories, Technical Guide 2. Second edition 2008, page 42.
5. Adriaan M. H. van der Veen, Juris Meija, Antonio Possolo, and D. Brynn Hibbert, IUPAC Recommendation: Guidelines for the use of atomic weights, Pure Appl. Chem. 2016; aop