The Uncertainty of Reference Standards—A Guide to Understanding Factors Impacting Uncertainty, Uncertainty Calculations, and Vendor Certifications

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Abstract

Certified solution standards are widely used in forensic toxicological, clinical/diagnostic, and environmental testing. Typically, these standards are purchased as ampouled solutions with a certified concentration. Vendors present concentration and uncertainty differently on their Certificates of Analysis. Understanding the factors that impact uncertainty and which factors have been considered in the vendor's assignment of uncertainty are critical to understanding the accuracy of the standard and the impact on testing results. Understanding these variables is also important for laboratories seeking to comply with ISO/IEC 17025 requirements and for those preparing reference solutions from neat materials at the bench. The impact of uncertainty associated with the neat material purity (including residual water, residual solvent, and inorganic content), mass measurement (weighing techniques), and solvent addition (solution density) on the overall uncertainty of the certified concentration is described along with uncertainty calculations.

Introduction

Reference materials are a critical element in the analytical testing laboratory. Certified reference materials (CRMs) are reference materials used as calibrators in quality control and method validation applications (1). CRMs are used to establish traceability to standard units of measure. Certified ampouled solution reference standards are widely used as calibrators and controls in the forensic toxicology, clinical/diagnostic, and environmental industries. In forensic and clinical settings, test results are used to support forensic investigations, therapeutic monitoring, and clinical decisions. Analytical results are only as good as the calibrators used in the analysis. Accuracy of quantitative results depends on robustness of the analytical method; preparation of samples and standards; and the quality, purity, and accuracy of the reference standard. A certified solution standard may be considered to be a CRM if the standard is characterized by a metrologically valid procedure for one or more specified properties, such as the solution concentration, and is accompanied by a certificate that provides the value of the specified property, its associated combined uncertainty, and a statement of metrological traceability. The certified solution standards prepared in Cerilliant's laboratories are manufactured to CRM standards. The stated unbiased solution concentration is reported with associated combined uncertainty and statement of traceability.

Traceability of the reference material and confidence in the property value (concentration) is critical to ensuring quality and accuracy of laboratory test results. ISO/IEC 17025 (2) and ISO guide 34 (3) require that laboratories maintain traceability and uncertainty of their reference materials as a key element of their quality systems. The stated concentration of a solution standard is usually accompanied by an uncertainty statement expressed as an expanded uncertainty in $\pm x$ units of concentration with a specified coverage factor ($C \pm ku_c$). Understanding the uncertainty of the concentration of reference standards is critical for laboratories seeking to ensure compliance with ISO/IEC 17025.

At Cerilliant, we have evaluated each step involved in the preparation of our certified solution standards and determined that the major contributing factors impacting uncertainty were 1. purity factor of the neat material, 2. mass measurement including weighing technique, balance selection and qualification, and weighing environment, and 3. solvent addition including density.

Methodology

Uncertainty is "a parameter associated with the result of a measurement that characterizes the dispersion of the values

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that could reasonably be attributed to the measurand." (4). For solution reference standards, whether purchased or prepared at the bench, the measurand in question is the concentration of the solution, usually specified in units of mass per volume.

The methods outlined in the EURACHEM/CITAC guide "Quantifying Uncertainty in Analytical Measurement" (1) were generally followed to document the process of propagating and reporting the uncertainty of the Certified Solution Standard concentration in this paper.

Definitions are as follows: measurement equation is used to define the relationship between the measurand (property being measured) and its required input values; standard uncertainty, u_i, represents an estimated standard deviation for the component (i); expanded uncertainty, U, is the confidence interval (±) around which the true value of the measurand will plausibly lie for a specified degree of confidence; coverage factor, k, is chosen based on the level of confidence associated with the expanded uncertainty (typically, $k = 2$ for 95% and $k = 3$ for 99% confidence).

Type A uncertainty refers to uncertainty evaluated using statistical distribution of a series of measurements; Type B uncertainty refers to other methods of uncertainty evaluation including assumed probability distributions based on tolerances, specifications, or experience.

Traceability is the property of a measurement result whereby it can be related to stated references usually through national or international standards through an unbroken chain of comparisons all having stated uncertainties. Traceability of measurements to international standard units is an important requirement of ISO guidelines.

In this study the method presented by Thomas Vetter and William Guthrie of NIST at the "Hands on Workshop on Evaluating Uncertainties for Chemical Analysis" presented at PITTCON 2007 (5,6) was used. The method facilitates use of spreadsheet type programs such as the Kragten spreadsheet (7). The use of this and similar spreadsheet models is also recommended in the EURACHEM/CITAC Guide Section 8.2.5 and Appendix E (1). The Kragten spreadsheet is useful in demonstrating the relative contribution of each component to the overall uncertainty, which can be valuable in identifying opportunities for process improvement.

Sequential perturbation was used to propagate each uncertainty component and calculate the combined standard uncertainty of all factors impacting the concentration value in the Kragten spreadsheet. Each component of uncertainty in the measurement equation is sequentially perturbed (varied) to determine the contribution of that uncertainty component on the overall uncertainty of the measurement. Uncertainty propagation using sequential perturbation can be generalized as shown in Equation 1 and Equation 2, where C is the final result of the measurement equation, x_i an input value to the measurement equation, u_i the uncertainty of the input x_i, u_C is the combined uncertainty of the measurement of C, and P is a perturbed value for C. The units of C and P are expressed in terms of the units of the measurement under study. For example in this paper, units of concentration are mass/volume.

$$C = f(x_1, x_2, x_3, \ldots x_n) \qquad \text{Eq. 1}$$

$$u_c = \sqrt{\delta C_1^{\,2} + \delta C_2^{\,2} + \delta C_3^{\,2} + \ldots \delta C_n^{\,2}}$$

$$\delta C_1 = P_1 - C,$$

$$\delta C_2 = P_2 - C, \text{ etc.}$$

$$P_1 = C(x_1 + u_1, x_2, x_3, \ldots x_n) \qquad \text{Eq. 2}$$

$$P_2 = C(x_1, x_2 + u_1, x_3, \ldots x_n), \text{ etc.}$$

The expanded uncertainty, U, is calculated using a coverage factor of $k = 2$ to approximate a 95% confidence interval for the solution standard concentration as shown in Equation 3.

$$U = 2u_C \qquad \text{Eq. 3}$$

Determination of Uncertainty for Solution Standard Preparation

It should be noted that the determination of uncertainty is specific to the processes and measurands under consideration. In this paper we present the determination of uncertainty specific to Cerilliant's manufacturing practices for the preparation of certified solution standards. The first step in the uncertainty determination process was to identify a measurement equation for solution standard preparation. This equation should comprise operations that go into preparation of a solution standard, and operations should include the preparer's approved procedures and process controls.

Every step involved in the preparation of our certified solution standards was evaluated to identify the measurement equation and key contributors to the uncertainty of the concentration. Equation 4 represents the specific measurement equation for the method of solution standard preparation employed, and Figure 1 demonstrates how the uncertainty of each of the individual elements feed into the combined uncertainty of the solution concentration. In this instance, concentration is expressed in units of mass per volume. The equation is based on a gravimetric method of preparation wherein

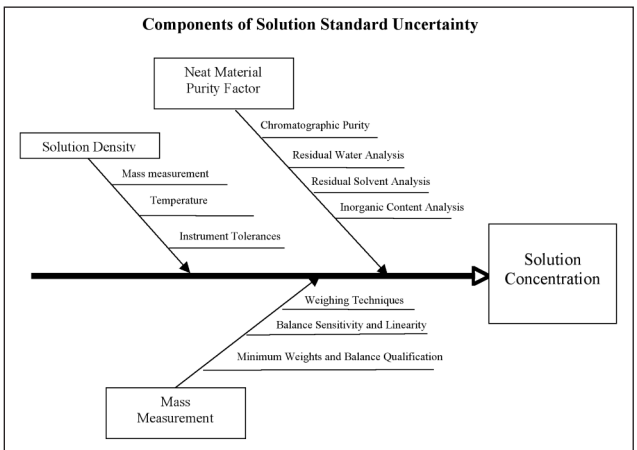

Figure 1. Components of solution standard uncertainty.

both solute and solvent are added gravimetrically and density of the solvent is used to convert units of mass to volume for the solution.

$$C = \frac{(m_{v+a} - m_v)dp}{(m_{f+s} - m_f)} \pm U \qquad \text{Eq. 4}$$

where C is concentration of solution (mass/volume), m_{v+a} represents mass of analyte + vial, m_v is mass of empty vial, m_{f+s} is mass of flask + solvent, m_f is mass of empty flask, d is density of solution, p is purity factor for the neat material, and U is the assigned combined expanded measurement uncertainty.

Purity factor of the neat material derives from measurement of chromatographic purity, residual water, residual solvent, and residual inorganic impurities. Mass measurement contributions to uncertainty encompass balance specifications and qualification, weighing technique, and weighing environment. Uncertainty related to mass measurement applies to each mass measurement term in the measurement equation. Solvent addition is performed gravimetrically with density measurement being the key contributor to solvent addition uncertainty.

Other factors evaluated for their contribution to the uncertainty of the solution concentration included the impact of the dispensing and ampouling process on concentration and homogeneity throughout the lot. Rigorous process controls are employed during the dispensing and ampouling processes. Verification of purity, concentration, and homogeneity during release testing demonstrated that the process controls are sufficiently robust to ensure little to no impact on uncertainty. These terms were, therefore, excluded from the uncertainty equation. Real-time stability testing with acceptance criteria identical to the original release testing is used to establish shelf life. Therefore, long-term stability is not a significant contributor to the uncertainty of the concentration.

Uncertainty associated with purity of the neat material u_p

In preparation of a solution reference standard, it is critical that the purity of the neat reference material be evaluated. Neat reference materials are typically assigned a purity factor or potency. This value should include corrections for chromatographic impurities and other residual impurities. The neat material purity factor is important because the analyte mass used in preparation of a solution reference standard from a neat reference material should be corrected for the purity factor of the neat analyte. Certificates of analysis (COAs) for neat reference materials should be carefully examined to ensure that the purity factor assigned was determined appropriately (i.e., that appropriate methods were used to determine chromatographic impurities and that the material was tested for other residual impurities).

In this study, certification of the neat material comprises determination of purity and impurities by a combination of analytical techniques to obtain a mass balance purity factor for the neat material. The mass balance result is used to accurately determine the amount of material to be weighed to achieve the desired concentration. The multiple method approach coupled with stringent specifications protects against random analytical error and is consistent with recommendations of ISO Guide 34 for certification of reference materials.

The purity of the neat material is determined chromatographically. Related substances such as residual water, residual solvent, and residual inorganic content are determined by specific techniques: Karl Fischer coulometry, gas chromatography (GC) headspace analysis, and ash analysis, respectively. The purity factor mass balance equation is shown in Equation 5. Each of these measurements has an associated uncertainty component that contributes to the overall uncertainty of the purity factor.

$$\text{Purity Factor} = \{[100 - (\text{wt\%OVI}) - (\text{wt\%H}_2\text{O}) - (\text{wt\%ROI})] \times (\text{ChromPurity}/100)\} \pm U \qquad \text{Eq. 5}$$

where wt%OVI is the weight percentage of residual solvents present in the neat material, wt%H_2O measures the weight percentage of water present in the neat material; wt%ROI represents the weight percentage of inorganic content in the neat material; ChromPurity is based on the chromatographic purity of the specified primary purity method, either GC or high-performance liquid chromatography (HPLC); and U equals the assigned combined expanded measurement uncertainty

The standard uncertainty of the chromatographic purity (u_{cp}) was determined by the purity specification for HPLC and GC–flame-ionization detection (FID) analyses requiring purity from a minimum of two different techniques to be within 0.5% of each other. This was considered a Type B error based on a uniform distribution pattern. In general, Type B errors may be estimated based on manufacturer tolerances or specifications for a test. The calculation is illustrated in Equation 6.

$$u_{cp} = 0.25\%/\sqrt{3} = 0.144\% \qquad \text{Eq. 6}$$

The standard uncertainty of residual solvent analysis, wt%OVI (u_{ovi}), was based on experimental determination of residual solvent in a 7-mg sample with all known analytes present at 0.015% (w/w). Results obtained from repeatability experiments using a validated headspace GC–FID residual solvent method were $u_{ovi} = 0.01746\%$ (w/w).

The uncertainty of residual water analysis, wt%H_2O, (u_{kf}) was based on replicate analyses of a sample containing 1% water by weight and a 15-mg sample size. Results were obtained from repeatability studies performed using the U.S. Pharmacopoeia Method 921 for Karl Fisher coulometry. The results were $u_{kf} = 0.03990\%$ (w/w).

The standard uncertainty of inorganic content analysis, wt%ROI (u_{roi}), was considered a Type B error based on uniform distribution and calculated as shown in Equation 7. As stated previously, Type B errors may be estimated based on manufacturer tolerances or specifications for a test.

$$u_{(\text{wt\%ROI})} = 0.4\%/\sqrt{3} = 0.231\% \text{ (w/w)} \qquad \text{Eq. 7}$$

The uncertainty values outlined were entered into a Kragten Spreadsheet using typical input values for each of the analytical test results. Examination of the Kragten revealed that the largest contribution to purity factor uncertainty arises from uncertainty of the inorganic content analysis with an approximate relative contribution of 70%. This arises primarily from

uncertainty in residual mass measurement of residue after ignition. The second largest contribution to purity factor uncertainty (~ 26%) is from the chromatographic purity analysis (based on specifications). Residual solvent and residual water contributed to < 5% of the overall uncertainty of the purity factor. Models were evaluated using a range of input values for the test results based on typical observations for neat material certifications. Figure 2 shows an example in which the input values for water, solvent, and ash are at the high end of values typically observed. The calculated uncertainty varied little with variations in the specific analytical input values for chromatographic purity, residual water, solvent, and ash. A single uncertainty value could therefore reasonably be employed as the contribution from neat material purity factor uncertainty in solution standard preparation. The relative standard uncertainty for the Purity Factor (u_P) was 0.292%. The Expanded % Uncertainty U (k = 2) was 0.58%.

A diagrammatic representation of the key elements of the uncertainty of the neat material purity factor is presented in Figure 3.

If an assay is used to determine purity factor (potency), then all elements associated with uncertainty of the assay must be evaluated, including uncertainty of the reference material potency and of the reference standard solution concentration and preparation; uncertainty of the assay sample solution preparation; and uncertainty of the analytical method.

Kragten Spreadsheet for Uncertaintyof the Purity Factor

Purity Factor = {[100 – (wt%OVI) – (wt%H_2O) – (wt%ROI)] x (ChromPurity/100)} ± U

Variable name, symbol	Value	units	Uncertainty source description	Reported uncer.	Type	Distribution	Factor to normalize	Standard uncer., u_i
Res. Solvent, *wt%OVI*	2.00	%w/w	QC Specification	0.01746	A	comb. std., k = 1	1	1.75E-02
Water Content, *wt%H2O*	4.00	%w/w	QC Specification	0.03990	A	comb. std., k = 1	1	3.99E-02
Inorganic Content, *wt%ROI*	1.00	%w/w	Outsourced Lab Specification	0.400	B	Uniform	0.577350269	2.31E-01
ChromPurity	99.00	%	Test Specification	0.250	B	Uniform	0.577350269	1.44E-01

		u(wt%OVI)	u(wt%H2O)	u(wt%ROI)	u(ChromPurity)	
		0.01746	0.03990	0.23094	0.14434	
Measurement Equation Inputs	Value					
wt%OVI	2.0000	2.01746	2.00000	2.00000	2.00000	
wt%H2O	4.0000	4.00000	4.03990	4.00000	4.00000	
wt%ROI	1.0000	1.00000	1.00000	1.23094	1.00000	
ChromPurity	99.0000	99.00000	99.00000	99.00000	99.14434	
Result	**92.0700**	**92.0527**	**92.0305**	**91.8414**	**92.2042**	
		-0.01729	-0.03950	-0.22863	0.13423	$c_i u_i$
u_c	0.268607	2.98785E-04	1.56033E-03	5.22720E-02	1.80188E-02	$(c_i u_i)^2$
k	2.000000	-9.90000E-01	-9.90000E-01	-9.90000E-01	9.30000E-01	c_i
U	0.53721		**0.583% $U_{relative}$, %**			**0.292% $u_{c\ relative}$, %**

RESULTS	
p (wt%)	92.0700
ν_{eff}	n/a
k	2
U (wt%)	0.53721

Figure 2. Kragten spreadsheet for uncertainty of the purity factor.

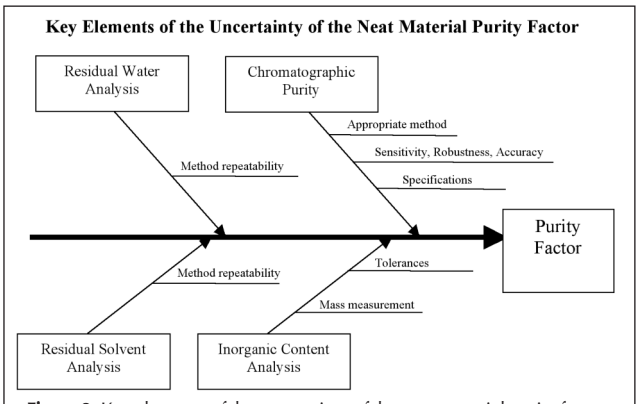

Figure 3. Key elements of the uncertainty of the neat material purity factor.

Uncertainty associated with preparation of the solution standard

As previously stated, uncertainty determinations are specific to the processes and methods used and measurements performed. Once neat material purity factor is established, the next step in preparation of a solution standard is the actual measurement and mixing of both the analyte and the solvent to form the solution. Solution standards prepared gravimetrically require addition of both analyte and solvent by weight. Addition of solvent by weight rather than volume allows control of variation in solvent volume due to density and temperature during preparation. Gravimetric preparation also provides greater traceability of weighing because balances are qualified and calibrated to SI units. Weigh tapes provide documented traceability. The uncertainty associated with gravimetric preparation utilizing appropriate weighing techniques is lower than that specified for class-A volumetric flasks when solutions are prepared by volumetric dilution. Thus, in a gravimetric method of preparation, mass measurement and solvent density terms are important contributors to uncertainty of the solution concentration.

Mass measurement considerations

Balance selection and qualification. Appropriate balance selection and qualification are critical to ensuring accuracy of the solution standard and can have a significant impact on the overall uncertainty. In our facility a full range of balances are used in solution standard preparation including 1, 3, 4, 5, 6, and 7-place balances (e.g., a 7-place balance measures to 0.0000001 g). Each balance has been fully qualified in its installed state and is calibrated semi-annually and adjusted weekly with NIST traceable weights. Balance performance is verified prior to use using NIST traceable weights. Balance selection and minimum weighings are outlined in standard operating procedures and were determined through the combination of manufacturer tolerances and repeatability experiments performed. Established minimum weighings for balances in our laboratories are shown in Table I and were calculated by Mettler Toledo during balance qualification to achieve USP-specified relative error of no more than (NMT) 0.1%. Improper balance selection can lead to high levels of uncertainty as demonstrated in Table II.

Weighing technique. Appropriate assignment of uncertainty of solution standard preparation must consider weighing technique in addition to balance selection and qualification. Accuracy of weighing can be influenced by specific factors such as the use of tongs versus gloved hands to handle vials and flasks, balance equilibration time, sample and solvent temperature, ambient temperature, vibrations, and movement of air. Air currents, drafts around the balance, and additional vibrational forces on the pan can significantly affect balance repeatability and lead to larger actual uncertainty. In one example, studies in our laboratory indicated that when gloved hands are used as

opposed to tongs for handling sample vials, uncertainty of mass measurement increased approximately 10-fold.

Uncertainty of the mass measurement u_m

The mass measurement uncertainty was determined from a combination of manufacturer-specified tolerances for sensitivity and linearity of the balances and repeatability experiments following specified weighing procedures. The values are proportional to the net mass being measured and are specific to the balance utilized. All balances in the study were manufactured by Mettler Toledo, and Mettler balance specifications were used (8).

Mass measurement uncertainty included several components. u_{sens} is uncertainty due to the balances sensitivity tolerance. The sensitivity tolerance includes the uncertainty of the balances built-in reference weight used for the internal calibrations. Balance manufacturer calibrations incorporate traceability to NIST SI units and their associated uncertainty in the sensitivity component.

u_{lin} is uncertainty due to non-linearity of the characteristic curve. u_{rep} is repeatability, which includes effects from, but not limited to, readability, drift, static, ambient drafts, thermal drafts, vibration, gross/net weight, eccentric loading, temperature stability, electromagnetic interferences/radio frequency interferences, weighing procedure, installation, tare container geometry, adsorption/absorption, and balance settings.

Repeatability was determined by tests of 20 replicate weighings conducted by multiple operators at various test loads and net weights on all balances used to prepare solution standards. To limit effects of internal balance settings, weighing parameters were predefined. Each balance had been qualified through an installation qualification and operational qualification and calibrated to NIST traceable weights semi-annually with weekly adjustments and verifications prior to use using NIST traceable weights. If a bias of > 0.1% relative error was observed, the balance was taken out of service for repair. The tests were designed to mimic typical weighing procedures used in preparation of a certified solution standard. Tared glass vials were used to simulate weighing of analytes and tared volumetric flasks to simulate weighing of solvents. The test loads covered the expected operating range of a given balance during typical production processes. The results from the repeatability tests were obtained as standard deviations and denoted s_p, the process standard deviation. This process standard deviation is an example of Type A uncertainty. The largest standard deviation, s_p, observed per process was used as the uncertainty of weighing repeatability, u_{rep}, to calculate the uncertainty value for weighing operations in Equation 8. Table III provides an example of results from repeatability experiments for different balances.

The root sum squared method was used to calculate the combined standard uncertainty as shown in Equation 8, where the square of the standard deviations represent the variances. The largest weighing uncertainty obtained for each balance tested is presented in Table IV.

$$u_m = \sqrt{u_{rep}^2 + u_{lin}^2 + u_{sens}^2} \qquad \text{Eq. 8}$$

To simplify assignment of uncertainties and eliminate the need to assign values on a per product basis, the largest single combined uncertainty (u_m = 0.035%) was selected to cover all weighing processes. This combined standard uncertainty value was used in the propagation of uncertainty in the concentration of solution standards.

The term for uncertainty from the weighing operation is applied to each weighing operation in the measurement equation (Equa-

Table I. Minimum Weighing Requirements

Balance	7-Place	6-Place	5-Place	4-Place
Balance resolution	0.0001 mg	0.001 mg	0.01 mg	0.1 mg
Minimum weighing	1 mg	3 mg	20 mg	125 mg

Table II. Importance of Balance Selection and Mass Uncertainty

	Mass Uncertainty	
Sample Mass	5-Place balance	4-Place balance
1 mg	8.0%	45.0%
10 mg	0.80%	4.5%
100 mg	0.080%	0.45%
1000 mg	0.0080%	0.045%

Table III. Repeatability Experiments for Different Balances Summary

Balance	XP6400 1 Place	XP1230S 3 Place	XP205 5 Place	XP56 6 Place	UMX2 7 Place
Process ID	1–10 L solvent	100–250 mL solvent	25 mg–10 g analyte	5 mg analyte	1 mg analyte
Approx. gross mass	1 kg	200 g	2.1 g	2.005 g	41 mg
Tare container	none	none	2-mL glass vial	2-mL glass vial	aluminum micro weigh pan
Ref./Net mass (g)	1000	200	0.1	0.005	0.001
Balance ID	C11074	C11090	C10901	C11088	C11087
Test point	grams	grams	grams	grams	grams
Mean	1000.000	200.0005	0.099995	0.0050023	0.00100193
Standard deviation (sp)	0.032	0.0012	0.000024	0.0000018	0.00000035
(% RSD)	0.00324%	0.00060%	0.02350%	0.03500%	0.0352248%

tion 4) for solution standard preparation, mass measurement of the analyte, and mass measurement of the solvent.

Addition of solvent/density uncertainty u_d. Solution standards are prepared gravimetrically to better control variables due to variation of solvent density (and therefore volume) with temperature and to provide traceability to SI units of mass. The mass of solvent to be added to the solution during standard preparation is calculated based on batch volume from the density at a specified temperature. The solvent mass is measured using appropriate qualified and calibrated balances and specified weighing techniques. Weigh tapes provide a record of traceability. Density is measured using a Mettler Toledo Densito 30PX density meter, which has a resolution of 0.0001 g/mL. For low concentration solutions (< 2 mg/mL), the solution density may be approximated using the density of the pure solvent. The uncertainty contribution of the density measurement was evaluated as a Type B component using the manufacturer's accuracy specification of ±0.001 g/mL, which was provided without specifying a level of confidence. Therefore, a uniform distribution was assumed to convert the value to a standard uncertainty value. The uncertainty component of the density was evaluated as shown in Equation 9.

$$u_d = 0.001/\sqrt{3} = 0.000577 \text{ g/mL} \qquad \text{Eq. 9}$$

Calculation of the combined standard uncertainty (u_C) and expanded uncertainty (U) of the solution standard concentration

Solution standard concentration uncertainty was calculated using the measurement Equation 4, and the uncertainty components were propagated using the sequential perturbation method of uncertainty propagation as explained in Equations 1–3. The mass measurement uncertainty term was applied to the mass measurements of the analyte and the solvent. The purity factor uncertainty was applied to the purity factor of the neat reference material. The density uncertainty was applied to the solvent density used in the calculation of solution concentration. The inputs used for the calculation are listed in Table V.

Inputs to the measurement equation for concentration could take on a wide range of values depending on batch volume, target concentration, solution density, and purity of analyte. Because of the diverse array of solution standards manufactured, it would be impractical to model every possible combination of measurement equation input values. Input values were, therefore, varied to provide models for concentration ranging from 1 to 5 mg/mL, batch volumes of 100–1000 mL, and varying purity factors. The calculations yielded uncertainty values for at least 99% of gravimetrically prepared solutions manufactured (Table VI). The varying input values were entered into a Kragten spreadsheet, and the uncertainty results over the given ranges were tabulated. The results were graphed and compared to determine whether batch sizes, concentration ranges, solution density, or value of purity factors need to be considered when assigning uncertainty, or if a single fixed value relative to the concentration may be applied to all gravimetrically prepared solutions.

Over the range of models tested (Table VI), the relative expanded uncertainties (expressed as percentage of concentration) varied little, reinforcing the value and importance of process controls employed. The highest value was 0.6291% and observed using a low density preparation of 0.6 g/mL. The lowest value observed in the range of models tested in Table VI was 0.6000%. Given this observation, it is reasonable to use a single relative uncertainty value for all solutions prepared within the variable ranges modeled. The relative standard uncertainty for solution standard concentration (u_c) was determined to be 0.315%. An expanded uncertainty (U) of 0.63% with a coverage factor (k) of 2 (relative to the prepared concentration) was assigned for all solution standards prepared gravimetrically following approved manufacturing procedures.

Trending in the models indicates negligibly small changes in uncertainty results when analyte mass, solvent mass, or adjustment factor values are varied. The largest influencing variable, while still relatively small, was solution density, where lower density solutions have increased uncertainty results.

Table IV. Standard Uncertainty for Weighing Operations by Balance Type (Based on Balance Specifications and Repeatability)

Balance Type and Model	Capacity	Net Mass	u_m	u_m Relative to Net Mass
1-place XP64001L	64,000 g	1 kg	0.04 g	0.004%
3-place XP1203S	1200 g	200 g	0.0012 g	0.0006%
5-place XP205	200 g	100 mg	0.000014 g	0.024%
6-place XP56	50 g	5 mg	0.0000018 g	0.035%
7-place UMX2	2 g	1 mg	0.00000035 g	0.035%

Table V. Input Values Used for Calculation of Solution Concentration Uncertainty

Input Description	Symbol	Value	Reported Uncer.	Units
Mass of vial	m_v	2	0.000035	g
Mass of vial+analyte	m_{v+a}	2.10000	0.000035	g
Mass of flask	m_f	50	0.021	g
Mass of flask+solvent	m_{f+s}	110.00000	0.021	g
Purity factor	p	100 (1)	0.00292	wt% (or g/g)
Solution density	d	0.6	0.000577	g/mL

Table VI. Range of Variables Modeled

Variable/Result Name	Lowest Modeled Value	Highest Modeled Value
Analyte mass	1 mg	5 g
Solvent mass	60 g	1.2 kg
Purity factor	100 wt% (1 g/g)	99 wt% (0.99 g/g)
Solution density	0.6 g/mL	1.2 g/mL
Batch volume	100 mL	1000 mL
Concentration	1 µg/mL	5 mg/mL

This is expected because the uncertainty component for density measurement is fixed, which results in increased relative uncertainty in density as the measured value decreases. A similar phenomenon is seen for laboratory balances and is the reason for establishing minimum sample weights to limit the relative measured mass uncertainty.

Use of the Kragten spreadsheet indicates the uncertainty of the purity factor to be the dominant source of the overall uncertainty in concentration, having an approximate relative contribution between 85 and 90% of the total uncertainty. The second largest contributing component is the uncertainty of the solution density, which ranged between 2.5 and 9.4% where the largest value is observed when modeling a solution density of 0.6 g/mL. As expected, in well-controlled weighing environments using controlled techniques, the mass measurements of solvent and analyte contribute the least to the overall uncertainty in concentration with a contribution of approximately 1.3% of the total uncertainty. According to guidelines for measurement uncertainty in the Eurachem/CITAC Guide (1), factors that contribute to less than one-third of the largest uncertainty contributor can generally be ignored; however, caution should be employed before eliminating a specific uncertainty component. The decision to include or eliminate a specific uncertainty contribution should include evaluation of the entire process, complexity of the process, all measurement inputs and impact of elimination of the component on other uncertainty contributors.

Discussion

There are several key points for consideration in the determination of uncertainty. Uncertainty is specific to the process and measurements under consideration. Development of an appropriate measurement equation is an important first step in determination of uncertainty.

Mass measurements should reflect laboratory specific procedures in addition to balance specifications. Although manufacturer specifications for balance uncertainty are quite low, these are typically obtained under ideal conditions using reference weights. Actual practice in the laboratory can vastly influence uncertainty related to weighing operations. The use of balance manufacturer specifications without evaluation of installed conditions and weighing practices provides an incomplete assessment of weighing uncertainty.

Gravimetric preparations provide greater control and traceability for solution standard preparation. If volumetric dilutions are required, the uncertainty associated with the preparation should be evaluated in detail and should account for errors arising from fluctuations of solvent density with ambient temperature and user error associated with visual read lines.

Thorough and appropriate characterization of the neat reference material is essential to determine an accurate mass balance purity factor, which is necessary for preparation of an accurate quantitative standard. The purity of the neat material and its associated uncertainty comprise a significant contribution to the overall uncertainty of the standard concentration. Key components of neat material certification are use of an accurate and robust method for determining purity and testing for impurities not detected by the primary purity method such as residual solvent, inorganic, and residual water content. Purity and residual impurity methods selected should be appropriate and accurate to the chemical properties of the material and provide resolution of related impurities.

Variations or changes to any component of the solution standard preparation process can impact uncertainty and requires reassessment of uncertainty values.

When a CRM is used, the uncertainty of the certified concentration should be incorporated into the uncertainty of the application or test in which the material is used.

In evaluating a vendor's COA, it is critical to understand what the uncertainty statement includes and/or omits. The following questions may be useful in this evaluation:

How was the uncertainty determined? What quality systems were used (such as ISO/IEC 17025 or ISO guide 34)?

What does the uncertainty value cover?

Is the uncertainty reported as an expanded uncertainty with a coverage factor? Are confidence intervals provided?

Is the neat material traceability and test data provided? Is purity of the neat material considered in the uncertainty of the standard preparation? Was the purity method appropriate for the compound, sufficiently robust, and repeatable?

What components are included in the Purity Factor assessment? Were residuals considered? What methods were used to determine these values?

Are environmental conditions such as temperature or density considered in the uncertainty statement?

Are balance and volumetric tolerances included those of the manufacturer alone or experimentally verified for the manufacturing process?

Conclusions

Accuracy of laboratory results require accurate references materials. Whether reference standards are purchased as certified solution standards or prepared at the bench from neat materials, understanding the accuracy of the reference and its associated uncertainty is critical to ensuring accuracy of test results. Determination of uncertainty in accordance with ISO/IEC Guide 17025 and ISO Guide 34 requires a thorough evaluation of all processes employed in the production of the reference standard, a determination of each factor's contribution to uncertainty, and development of uncertainty budgets for the process. The process of determination of uncertainty highlights critical points in the standard preparation process and the criticality of the neat material certification. A thorough evaluation of uncertainty budgets can result in better process controls to reduce uncertainty and allows for an accurate understanding of the quality of the reference standard produced.

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