The Uncertainty of Reference Standards

A Guide to Understanding Factors Impacting Uncertainty, Uncertainty Calculations and Vendor Certifications

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Analytical Reference Standards

Introduction

Analytical results are only as good as the calibrators used in the analysis.

Certified ampouled solution reference standards are widely used in the forensic/toxicology, clinical/diagnostic, and pharmaceutical industries to support forensic investigations, therapeutic monitoring, and clinical decisions.

Accuracy is dependent on: robustness of the analytical method, preparation of samples and standards, & the quality, purity and accuracy of the reference.



Uncertainty

Uncertainty is *"a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand."**

*International Vocabulary of Basic and General Terms in Metrology

For solution reference standards, whether purchased or prepared at the bench, the measurand in question is the concentration of the solution (specified in units of mass per volume).

Understanding uncertainty variables is important for laboratories seeking to comply with ISO/IEC 17025 requirements and for those preparing reference solutions from neat materials at the bench.



Uncertainty is an estimation

Factors Impacting Concentration

- Neat material purity & homogeneity
- Solution preparation techniques/methods
- Weighing equipment & procedures
- Pipettes and flasks
- Solution storage, handling, & homogeneity



Cerilliant's Approach to Reference Solution Preparation

- Complete characterization of neat materials
- Qualified and controlled weighing processes
- Gravimetric addition of solvent
- Sealed into ampoules under argon
- Analytical verification of concentration, purity, & homogeneity
- Shelf life determined through real-time stability studies

Methodology for Uncertainty

Kragten Spreadsheets and differential perturbation

W. Guthrie, T. Vetter. "Hands-on Workshop on Evaluating Uncertainties for Chemical Analysis" Gaithersburg, MD: National Institute of Science and Technology, PITTCON 2007.

Cerilliant's Measurement Equation

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

Where: C = Concentration of solution (mass/volume)

m_{v+a} = mass of analyte + vial

m_v = mass of empty vial

m_{f+s} = mass of flask + solvent

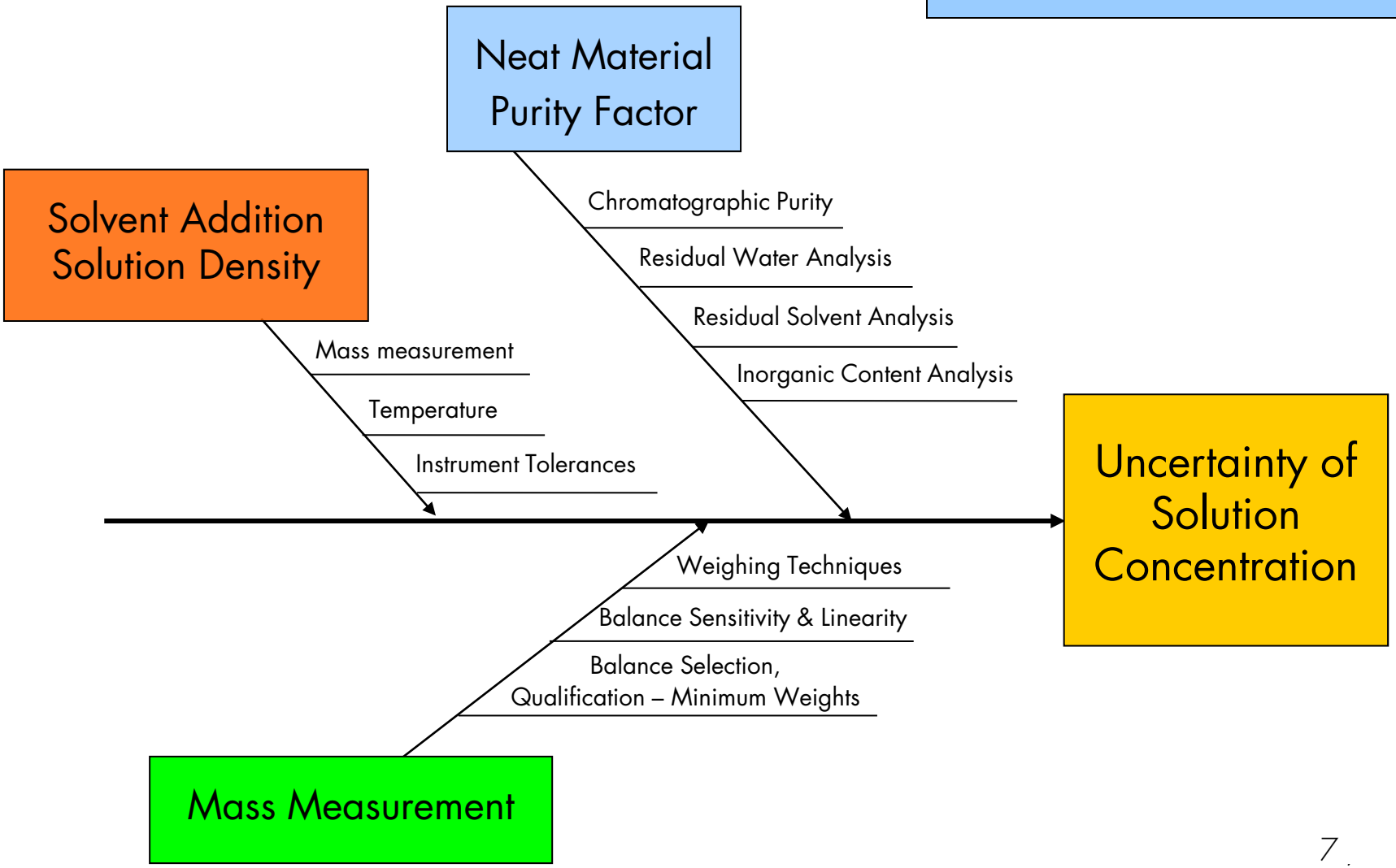
m_f = mass of empty flask

d = density of solution

U = the assigned expanded measurement uncertainty

Factors Impacting Uncertainty of Solution Standard Preparations

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Uncertainty Associated with Purity of Neat Material

- Is the neat material certification adequate for reference standard use? – Includes **Purity and Residuals**?
- The Cerilliant **Purity Factor** mass balance equation – often referred to as "potency" on vendor COA's – is **used to calculate** the amount of material needed to achieve **accurate concentration** of the solution standard.

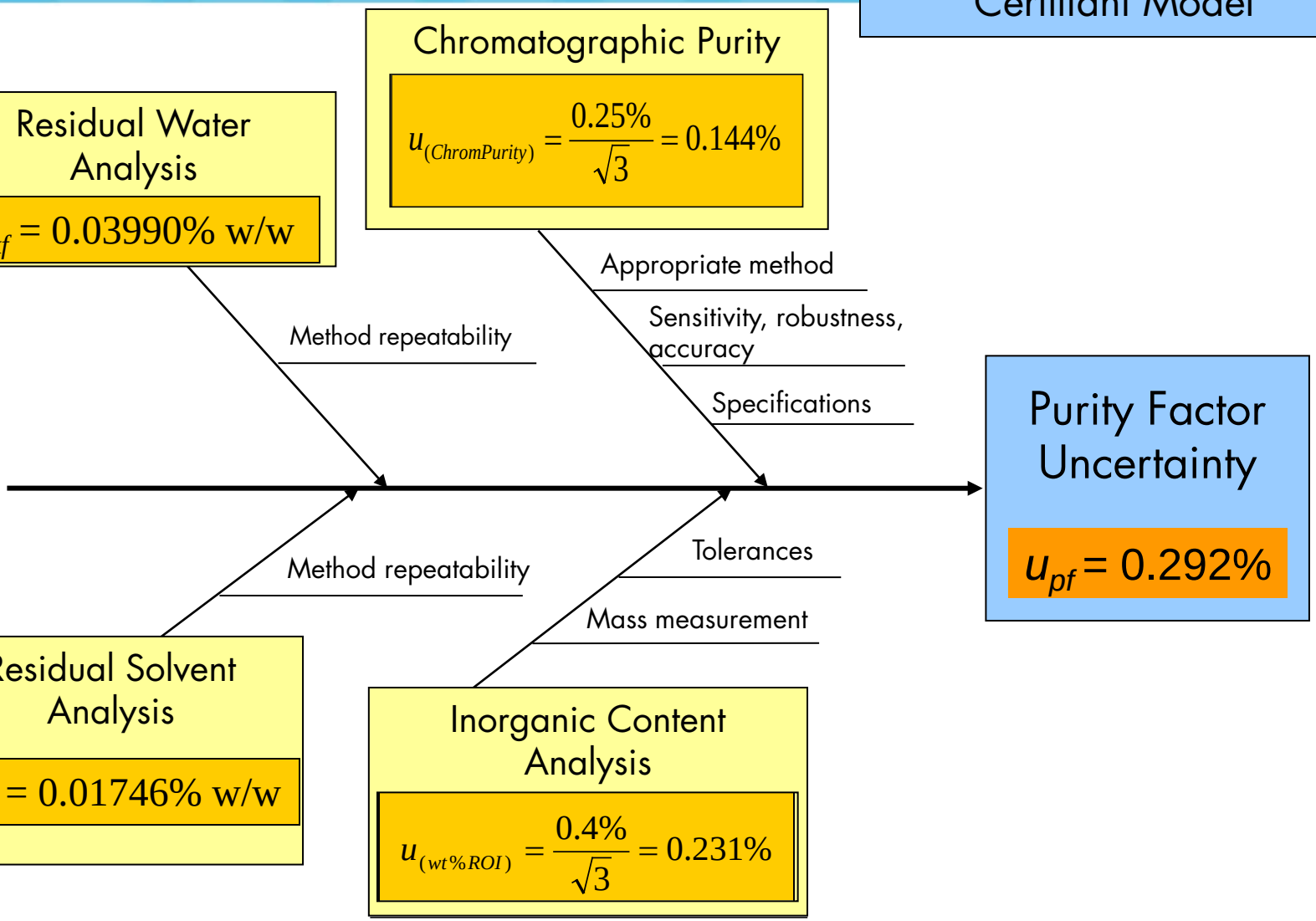
$$PurityFactor = \left[[100 - (wt\%OVI) - (wt\%H_2O) - (wt\%ROI)] * \frac{ChromPurity}{100} \right] \pm U$$

wt%OVI: the weight percentage of residual solvents present in the neat material.
wt%H2O: the weight percentage of water present in the neat material.
wt%ROI: the weight percentage of inorganic content in the neat material.
ChromPurity: based on the chromatographic purity of the specified primary purity method, either GC or HPLC.
U = the assigned expanded measurement uncertainty

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Key Elements of Uncertainty
Neat Material Purity Factor

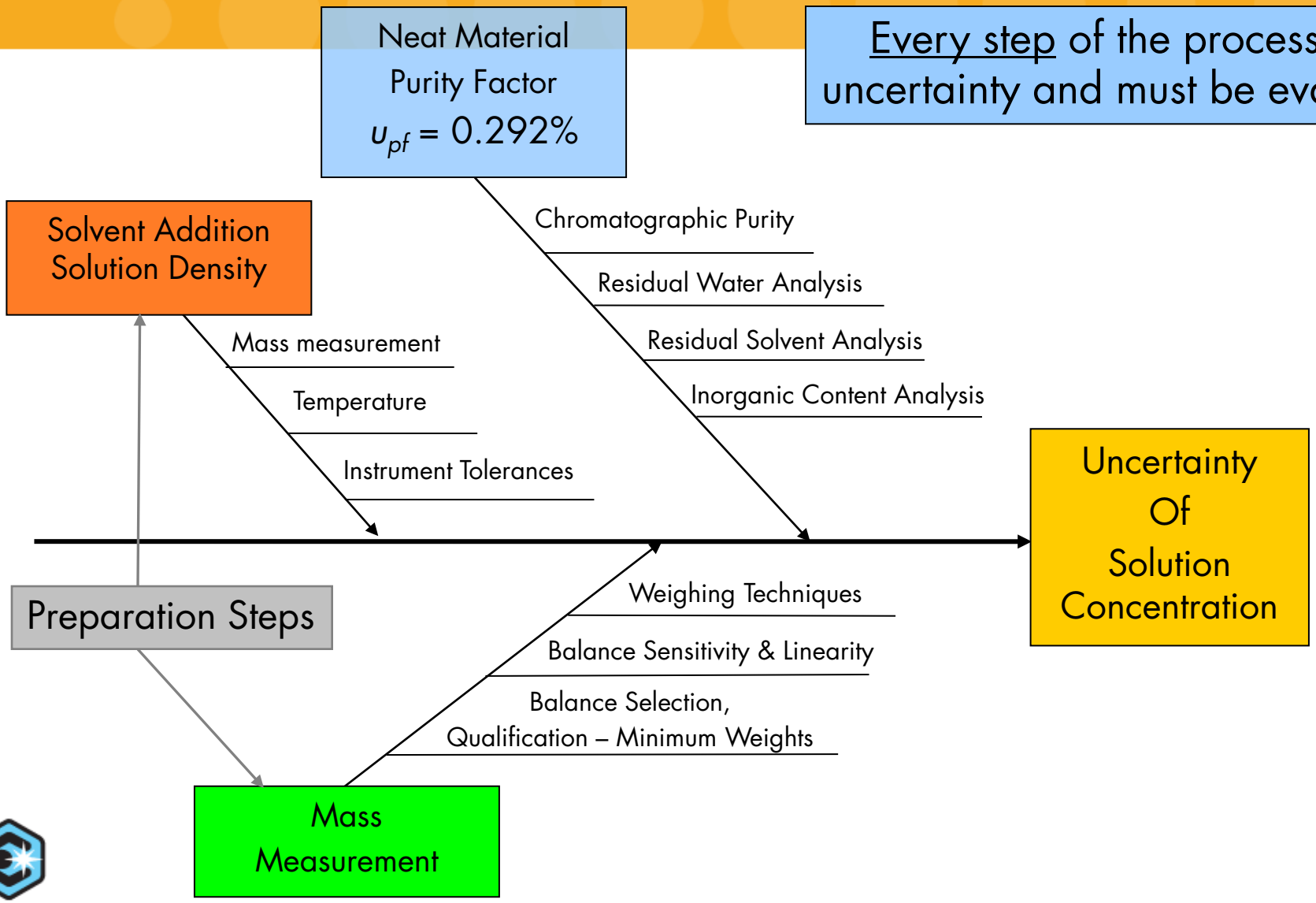
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Purity Factor Considerations

- Appropriate methods for purity determination
- Quantitation of residual impurities
  - Water
  - Solvent
  - Inorganics
- Use of a mass balance equation
- Stability – purity and residuals

Factors Impacting Uncertainty of Solution Standard Preparations



Uncertainty of the Mass Measurement u_m

Factors impacting mass measurement uncertainty

- Mass measurement uncertainty included the following components:
  - u_{sens} Uncertainty due to the **balance's sensitivity tolerance**. The sensitivity tolerance includes the uncertainty of the balance's built-in reference weight used for the internal calibrations.
  - u_{lin} Uncertainty due to **non-linearity of the characteristic curve**.
  - s_p **Repeatability** includes effects from readability, drift, static, ambient drafts, thermal drafts, vibration, gross/net weight, eccentric loading, temperature stability, EMI/RFI, **weighing procedure**, installation, tare container geometry, adsorption/absorption, and balance settings.

Repeatability is specific to YOUR environment, YOUR balances, and YOUR weighing technique

Mass Measurement Considerations

Appropriate balance selection and qualification are critical to ensuring accuracy of the solution standard and can have a significant impact on the overall uncertainty

- Improper balance selection can lead to high levels of uncertainty

Importance of Balance Selection and Mass Uncertainty		
Sample Mass	Mass Uncertainty	
	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%

- Minimum weighings established to achieve USP specified minimum relative error of NMT 0.1%.

Cerilliant 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

Mass Measurement Considerations

Weighing Technique can significantly influence uncertainty

- 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:
  - tongs vs. gloved hands
  - balance equilibration time
  - sample and solvent temperature
  - ambient temperature
  - vibrations
  - movement of air

For Example:

Cerilliant studies indicate that when gloved hands are used as opposed to tongs for handling sample vials, uncertainty of mass measurement increased approximately 10 fold.

- Air currents, drafts around the balance, and additional vibrational forces on the pan can significantly affect balance repeatability.



Uncertainty of Mass Measurement u_m

Repeatability experiments are used to determine preparer specific uncertainty for weighing operations

Repeatability Experiments for Cerilliant Preparations Using Different Balances					
Balance	XP6400	XP1230S	XP205	XP56	UMX2
	1 Place	3 Place	5 Place	6 Place	7 Place
Process Scale	1-10 Liters	100-250 mL	25 mg–10 g	5 mg	1 mg
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
Mean	1000.000	200.0005	0.099995	0.0050023	0.00100193
Standard Deviation	0.032	0.0012	0.000024	0.0000018	0.00000035
%RSD (s_p)	0.00324%	0.00060%	0.02350%	0.03500%	0.0352248%



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Uncertainty of the Mass Measurement u_m

- The largest standard deviation observed in repeatability tests per process was used to calculate the uncertainty value for weighing operations

  (u_m = 0.035%)

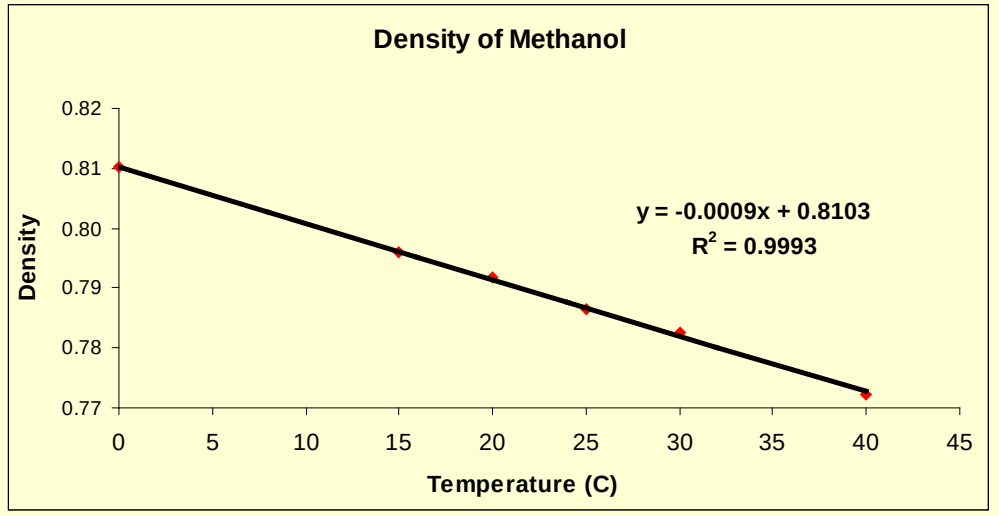
$$u_m = \sqrt{s_p^{\,2} + u_{lin}^{\,2} + u_{sens}^{\,2}}$$

- The term for uncertainty from the weighing operation is **applied to each weighing operation** in the measurement equation for solution standard preparation, mass measurement of the analyte and mass measurement of the solvent.

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

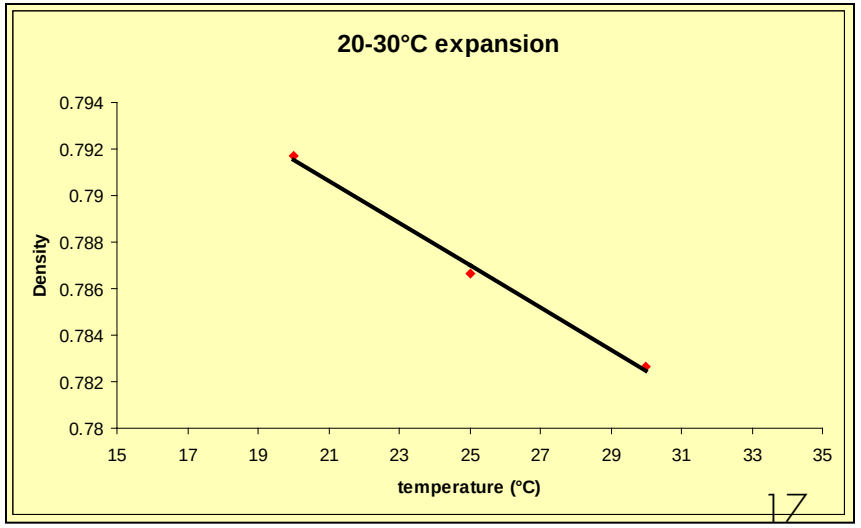
Solvent Addition Temperature vs. Density

Change in density with temperature can affect volumetric preparation of a solution but can be controlled by gravimetric addition of solvent



0.57% difference in concentration when prepared volumetrically at 20° vs. 25°C

Bench preparation of sample and reference on different days may create variability due to density change



Source: Handbook of Thermophysical and Thermochemical Data, CRC Press

Addition of Solvent / Density Uncertainty u_d

- Gravimetric addition controls for variation of solvent density (and therefore volume) with temperature
  - traceability to SI units of mass.
  - Solvent mass required is calculated from the density
  - Solvent is added by weight
  - Solution mass is converted back to volume by dividing the mass by the density.

    (For low concentration solutions (< 2 mg/mL), the solution density is approximated using the density of the pure solvent)

- Density is measured using a Mettler Toledo Densito 30PX density meter which has a resolution of 0.0001 g/mL.
  - The uncertainty component for density was estimated based on instrument tolerances

$$u_d = \frac{0.001}{\sqrt{3}} = 0.000577 \text{ g/mL}$$

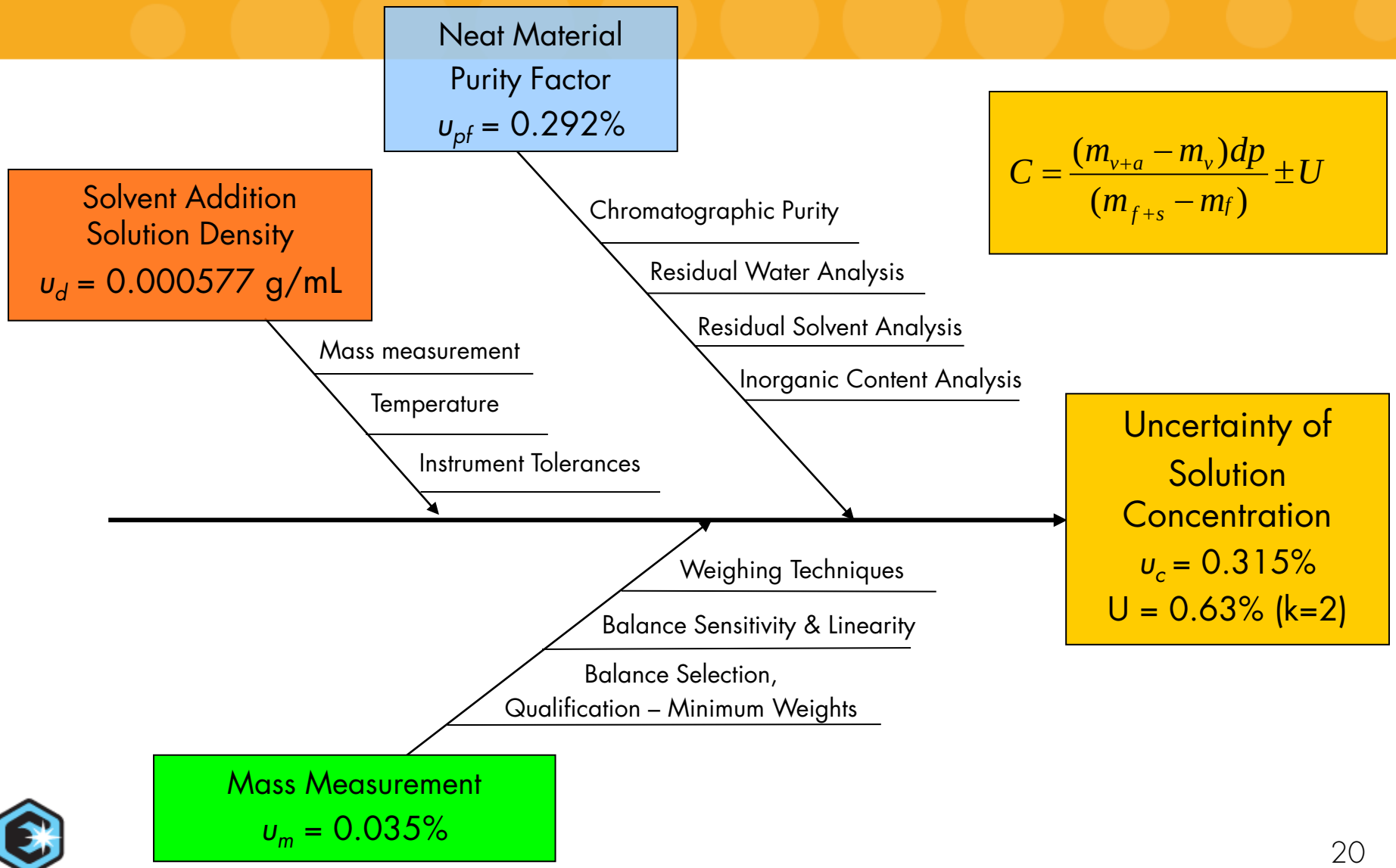
Calculation of the Combined Standard Uncertainty (u_c) and Expanded Uncertainty (U) of the Solution Standard Concentration

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
Adjustment factor	1	1.01
Solution density	0.6 g/mL	1.2 g/mL
Batch volume	100mL	1000mL
Concentration	1 µg/mL	5 mg/mL

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. Input values were, therefore, varied to provide models which yielded uncertainty values for at least 99% of gravimetrically prepared solution.

Over the range of models tested the relative expanded uncertainties varied little, reinforcing the value and importance of process controls employed.

Uncertainty of Solution Standard Preparations



Considerations

4 Points of Consideration

1. Thorough characterization of the neat material is essential to determine an accurate mass balance purity factor.
   - Characterization of the neat material should be appropriate for use
   - Should include both purity and residuals
   - Uncertainty contributions from neat material certification are significant

2. Actual practice in the laboratory can vastly influence uncertainty related to weighing operations.
   - Balance manufacturer specifications are insufficient
   - Repeatability assessment must be included

3. Gravimetric preparations provide greater control and traceability for standard preparation.
   - Volumetric dilutions should account for errors arising temperature/density effects and user error associated with visual read lines.

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



Considerations

Evaluating Certificates of Analysis

- 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?
- If the standard was diluted from a stock solution, does the uncertainty include uncertainty of the secondary dilution?



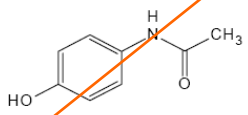
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Certificate of Analysis

Cerilliant Quality
ISO GUIDE 34 ACCREDITED
ISO/IEC 17025 ACCREDITED
ISO 9001:2000 CERTIFIED

Acetaminophen

4-Acetamidophenol



Catalog Number:	A-064
Solution Lot:	FN022009-02
Expiration Date:	February 2014
Solvent:	Methanol
Volume per Ampule:	Not less than 1 mL
Storage:	Protect from air and light, refrigerate or freeze.
Intended Use:	For laboratory use only. Not suitable for human or animal consumption.

- Expiration Date has been established through real time stability studies.
- Ampules are overfilled to ensure a minimum 1 mL volume fill. We advise laboratories to use measured volumes of this standard solution before diluting to the desired concentration.

Component	Chromatographic Purity	Concentration
Acetaminophen	99.9%	1.000 ± 0.006 mg/mL

- Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the purity factor, material density, and mass.
- Concentration is corrected for chromatographic purity, residual water, residual solvents and residual inorganics.

Solution Standard Verification and Homogeneity

Standard Solution	Lot Number	Verified Concentration (mg/mL) Actual Results	Verified Concentration (mg/mL) Acceptance Crtieria	%RSD - Homogeneity Actual Results	%RSD - Homogeneity Acceptance Crtieria
New Lot	FN022009-02	0.998	± 3%	0.8	≤ 3%
Previous Lot	FN092707-01	0.996	± 3%	0.4	≤ 3%

- Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration curve.
- Homogeneity of the New Lot is ensured through rigorous production process controls statistically analyzed to evaluate risk and verified by analysis. The % RSD of samples pulled from across the lot demonstrate homogeneity of the New Lot.
- The % RSD of the Previous Lot represents variability of the analysis.

Traceability

- Gravimetrically prepared using qualified balances calibrated semi-annually by Mettler Toledo using NIST traceable weights. Calibration verification performed weekly and prior to each use utilizing NIST traceable weights. Each balance has been assigned a minimum weighing by Mettler Toledo taking into consideration the balance and installed environmental conditions to ensure weighing complies with USP tolerances of no more than 0.1% relative error.
- Concentration is verified against an independently prepared 4-point calibration curve gravimetrically prepared using balances calibrated to NIST

Cerilliant certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration/retest date.

ACLASS

Lara Sparks, Quality Assurance Director

March 18, 2009
Date

Cerilliant Corporation 811 Paloma Drive, Suite A, Round Rock, TX 78665 800-848-7837 / 512-238-9974

Solution Purity is verified chromatographically post ampouling to ensure no degradation or contamination

Concentration & Uncertainty of the gravimetric preparation expressed as:

1.000 ± 0.0006 mg/mL

Description of Cerilliant's Uncertainty value & confidence interval:

"Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k = 2 and has been calculated by statistical analysis of our production system and incorporates uncertainty of the purity factor, material density, and mass".

Analytical Verification of Concentration & Homogeneity

Gravimetrically prepared concentration is verified analytically. Acceptance criteria incorporates variability of the analysis. Homogeneity is verified analytically by analyzing ampoules pulled from across the lot.

Traceability Statement describing traceability to SI units

"Gravimetrically prepared using qualified balances calibrated semi-annually by Mettler Toledo using NIST traceable weights. Calibration verification performed weekly and prior to each use utilizing NIST traceable weights. Each balance has been assigned a minimum weighing by Mettler Toledo taking into consideration the balance and installed environmental conditions to ensure weighing complies with USP tolerances of no more than 0.1% relative error".

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Standard Solution Assay Parameters

Analysis Method:	HPLC/UV
Column:	Luna 5µ C_{18}, 4.6 x 250 mm
Mobile Phase:	Methanol::Water (50::50)
Flow Rate:	1.0 mL/min
Wavelength:	246 nm

Calibration Curve

Calibration Curve:	Linear Regression
Number of Points:	4
Linearity (r):	0.999

Neat Material Data

Compound Name:	Acetaminophen	**Chemical Formula:**	$C_8H_9NO_2$
Compound Lot:	PN061507-01	**CAS Number:**	103-90-2
		Molecular Weight:	151.16

Neat Material Characterization Summary

Analytical Test	Method	Results
Primary Chromatographic Purity by HPLC/PDA Analysis	SP10-0102	99.9%
Secondary Chromatographic Purity by GC/FID Analysis	SP10-0101	99.9%
Identity by GC/MS Analysis	SP10-0105	Consistent with Structure
Identity by ^{1}H-NMR Analysis	SP10-0116	Consistent with Structure
Residual Solvent Analysis by GC/FID Headspace	AM1087	0.00%
Residual Water Analysis by Karl Fischer Coulometry	SP10-0103	< 0.2%
Inorganic Content by Microash Analysis	Outsourced	< 0.1%
Purity Factor		99.90%

- Primary purity is calculated as the average of two independently performed analyses utilizing two different methods. Acceptance criteria requires the purity values to be within 0.5% of each other.
- The primary chromatographic purity value is used to calculate the Purity Factor.
- A secondary chromatographic purity method is utilized as a control.
- Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100].
- Purity factor does not include adjustment for chiral and/or isotopic purity.

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Neat Material Characterization Summary and Purity Factor Assignment

Shows detail of all analyses performed and results – allows confirmation of Purity Factor variables considered

Acknowledgements and References

Acknowledgements:

- Ning Chang PhD, Isil Dilek PhD, Kevin Gates, Huahua Jian PhD, Sherri Pogue , Kristine Waddell

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science, smarter.®

- Cerilliant Quality
- ISO GUIDE 34
- ISO/IEC 17025
- ISO 9001:2000
- GMP/GLP