Preparation and Uncertainty of Pharmaceutical Solution Standards for Use in Drinking Water Analysis

AUTHORS

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Abstract

Contamination of drinking water supplies with pharmaceuticals is of increasing concern around the U.S. Trace levels present a potential threat to health and the environment. Identification and quantitation of pharmaceutical impurities in effluent and drinking water is an emerging focus for testing laboratories and is addressed in EPA methods 1694 and 1698.

The accuracy of reference standards used in these tests is critical. High purity neat and certified solution standards are available from several sources.

EPA method 1694 states "If the chemical purity is 98% or greater, the weight may be used without correction to calculate the concentration of the standard." However, this statement does not consider nonchromatographic impurities such as residual solvent, water, or trace inorganics; uncertainty associated with purity of the neat material; or how these components directly affect concentration of the analytical solution. Certain neat materials contain considerable levels of residual solvent due to the compound's crystal structure, functional groups, or solvent of crystallization. Changes in residual water content of a neat material over time can significantly impact the concentration of analytical solutions prepared from the neat in the testing laboratory. Ampouled Certified Solution Standards offer a viable and accurate alternative.

Different vendors present concentration and uncertainty information in different ways on their certificates of analysis. Understanding the certificate of analysis – what is and is not included in the reported uncertainty – and factors that impact preparation of a certified solution standard are critical to understanding the accuracy and uncertainty of the standard and the impact on testing. Understanding these variables is also important for labs seeking to comply with ISO 17025 requirements.

The preparation, uncertainty, and stability of Cerilliant Certified Snap-N-Shoot® Solution Standards for EPA Methods 1694 and 1698 will be presented. The impact of uncertainty of the neat material purity, residual water, residual solvent, and inorganic content on preparation of the solution standard and stability will be demonstrated. Comparison and explanation of uncertainty statements from a variety of certificates of analysis will also be presented.

REFERENCE STANDARDS ARE CRITICAL TO THE QUANTITATION OF PHARMACEUTICALS IN DRINKING WATER

With EPA method detection limits and minimum quantitation levels often at low concentrations, the presence of residual solvent or water, or slight changes in these components over time will impact accuracy of the standard preparation and concentration of the solution. This effect becomes most critical for quantitating results at the lower limits of the method.

Results are only as accurate as the reference! Accuracy depends on robustness of the analysis and quality of the reference.

Certified Spiking Solution & Solution Standards, when properly prepared, ensure accurate, consistent, and reliable results in quantitative analysis of drinking water and other environmental samples for contaminants and eliminate issues with changes in the neat material over time.

Proper design, preparation, and certification of solution standards are important for achieving accuracy, consistency, and long term stability.

Cerilliant®
Analytical Reference Standards

REFERENCE STANDARD DESIGN & PREPARATION

ACCURACY, CONSISTENCY, & STABILITY ACHIEVED THROUGH PROPER DESIGN & PREPARATION

Considerations During Solution Preparation:

- Neat material characterization including residuals
- Accuracy of weighing operations
- Accuracy of solvent addition
- Packaging & storage
- Assessment of shelf life

Certification demonstrates that a product is accurate, consistent, and stable and conforms to stated specifications

NEAT MATERIAL CHARACTERIZATION RESULTS → PURITY FACTOR

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.

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

wt%OVR: the weight percentage of residual solvents present in the neat material.

wt%H_2O: the weight percentage of water present in the neat material.

wt%ROI: the weight percentage of inorganic purity of the specified primary purity method, either GC or HPLC.

U: the assigned expanded measurement uncertainty.

NEAT MATERIAL CHARACTERIZATION

Complete & accurate characterization of neat material is essential to accuracy of the solution

Characterization of neat materials should include

- Purity and impurities
  - Chromatographic purity
  - Method appropriate for resolution of impurities and related compounds (LC, GC)
- Residual components
  - Water, solvent, inorganic content
  - Analytical techniques: KF, headspace, micro-ash/ROI
- Verification of identity (NMR, FTIR, MS)

Characterization Considerations

- Are vendor certified values complete, accurate, and reliable?
- Reliability/repeatability of the method?
- Does the vendor provide uncertainty on the purity factor (potency)?

IMPACT OF RESIDUAL CONTENT

Chromatographic Purity – just the beginning...

- Residual Water & Hygroscopicity
  - A neat reference material may contain residual water and/or absorb moisture over time despite high chromatographic purity.
  - Absorption of moisture over time will impact subsequent weighing of the material and must be re-evaluated prior to use in quantitative applications.
- Residual Solvent
  - A neat reference material may contain residual solvent such as a solvent of crystallization despite high chromatographic purity.
  - Residual solvent values should remain stable over time when properly stored.
- Trace Inorganic Content
  - Due to the synthetic route, extraction process, or purification procedure, many materials may contain trace inorganics.

Residuals must be included in the purity factor for quantitative applications.

IMPACT OF RESIDUAL CONTENT ON NEAT MATERIAL PURITY FACTOR

Compound	Chrom. Purity (%)	Residual Solvent Content (%)	Trace Inorganic Content (%)	Residual Water Content (%)	Purity Factor for Quantitative Use (%)
Albuterol	**99.9**	0.04	N/A	1.33	**98.57**
Ranitidine HCl	**99.5**	0.87	0.13	None Detected	**98.47**
Oxazepam Glucuronide	**99.9**	None Detected	2.37	8.96	**88.58**
Morphine-3/6-D-Glucuronide 1/2007	**99.8**	None Detected	< 0.1%	3.36	**96.45**
Morphine-3/6-D-Glucuronide 4/2009	**99.6**	1.38	< 0.1%	3.11	**95.10**
	99.6	1.38	< 0.1%	7.23	**91.00**

Without full characterization of the neat material, significant error may be introduced into the concentration of the reference solution

WEIGHING ACCURACY

Larger weighings - more accurate

- 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 should be established to achieve minimum relative error.
- Cerilliant specifies minimum weighings to achieve USP tolerances of ≤0.1% relative error.

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

- Accuracy of weighing can be influenced by:
  - Tongs vs. gloved hands
  - Balance equilibration time
  - Sample and solvent temperature
  - Ambient temperature
  - Vibrations
  - Movement of air
- Air currents, drafts around the balance, and additional vibrational forces on the pan can significantly affect balance repeatability.

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.

Balance environment & weighing technique can significantly influence reference accuracy

GRAVIMETRIC APPROACH ADD SOLVENT BY WEIGHT

- Target solvent weight is calculated from target volume by adjusting for density. Actual solvent weight is calculated back into volume to report concentration in mg/mL or other mass/volume units of measure.
- Accuracy of balance vs. volumetric flask
- Impact of temperature on density
- Subjectivity of visual fill line
- Weigh tapes provide traceability to SI units
- Weigh tapes provide audit trail

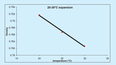
Method	Batch Size		
	10mL	100mL	1000 mL
Volumetric flask standard error	0.20%	0.08%	0.03%
Source: ASTM E288-03, Standard specification for laboratory glassware, 2003			
Analytical balance uncertainty	5 Place	5 Place	1 Place
Balance Type	0.001%	0.0001%	0.009%
Typical values per Mettler Toledo			
Values established by Cerilliant based on typical values by Mettler and Cerilliant weighing SOPs	0.0036%	0.00125%	0.009%

Use of a high quality, qualified balance has lower error than Class-A volumetric flask

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

- Ensures lot-to-lot consistency
- Differences between sample temperature and solvent temperature
- Consistency between sample and reference or calibrators and controls prepared on different days or in different environments



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

Source: Handbook of Thermophysical and Thermochemical Data, CRC Press

DISPENSING & PACKAGING SNAP-N-SHOOT® /SNAP-N-SPIKE™ FORMAT

- Flame sealed under argon into amber ampoules
- Provides protection from changes to the neat material such as issues with hygroscopicity
- Process controls ensure
  - Consistency of volume dispensed
  - Homogeneity from vial to vial and across the lot
  - No contamination
  - No degradation

Provides protection from hygroscopicity, degradation, evaporation, & contamination
Promotes Stability

SOLUTION STABILITY

ASSESSMENT OF SOLUTION STABILITY

Expiration is established through real-time stability studies. Solution purity and concentration are re-evaluated at multiple intervals.

Compound/Solvent	Age of Stability Sample	Purity		Analyzed Concentration	
		Original	Stability Interval	Original	Stability Interval
Acetaminophen/methanol (mg/mL)	5.2 years	99.1%	99.8%	0.967	1.003
Fluoxetine HCl/methanol (mg/mL)	6.3 years	99.0%	99.8%	0.992	1.000
Codeine/methanol (mg/mL)	5.5 years	99.9%	99.4%	0.989	0.995
Caffeine/methanol (mg/mL)	4.6 years	99.8%	99.7%	0.992	1.061
Testosterone/acetonitrile (mg/mL)– studies on-going	1.8 years	99.8%	99.2%	0.991	0.992

Concentration acceptance criteria for each of the examples = ± 3% and incorporates variability of the analysis.

ACETAMINOPHEN

Stability established at 5.2 years

Manufacture Date	Test Date	Analyzed Concentration (mg/mL)	Solution Purity
12/2003	2/2009	1.003	99.8%

CODEINE

Stability established at 5.5 years

Manufacture Date	Test Date	Analyzed Concentration (mg/mL)	Solution Purity
10/2002	2/2009	1.00	99.8%

GEMFIBROZIL

Accelerated Stability Study

Storage Condition/ Test Interval	Gemfibrozil solution purity(%)
Initial (t=0)	99.9
Freezer (-5 to -25°C)	
1 week	99.9
2 weeks	99.9
4 weeks	99.9
Refrigerate (2 to 8°C)	
1 week	99.9
2 weeks	99.9
4 weeks	99.9
Ambient (18 to 30°C)	
1 week	99.9
2 weeks	99.9
4 weeks	99.9
Elevated (40°C)	
1 week	99.9
2 weeks	99.9
4 weeks	99.9

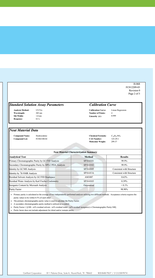
Samples were stored at the following storage conditions: Refrigerate, Freezer, Ambient and Elevated Temperature and analyzed at 1, 2 and 4 weeks.

CERTIFICATION & UNCERTAINTY


Cerilliant

Certificate of Analysis

Hydrocodone



- **Expiration/Retest Date**
  Expiration Dates are established through real-time stability studies. Retest Dates are assigned to ensure products are continually evaluated during the expected period shelf-life. Refer to Expiration/Retest policy for additional information.
- **Solution Purity**
  Extensive process controls are employed during manufacturing to ensure no degradation or contamination occurs. Analytical verification of solution purity post ampouling provides absolute confirmation.
- **Concentration & Uncertainty**
  Concentration of the gravimetric preparation expressed as 1.000 + 0.0006 mg/mL
  Represents the actual concentration (not theoretical) based on material weighings and material Purity Factor
- **Uncertainty**
  The Uncertainty Statement provides details of what standards were used to develop the uncertainty value, the confidence interval, the coverage factor and the processes or steps incorporated in the uncertainty value.
  "Uncertainty of the concentration is expressed as an expanded uncertainty in accordance with ISO 17025 and ISO 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**
  The gravimetrically prepared concentration is verified analytically by comparison to an independently prepared calibration solution. Lot-to-lot consistency is demonstrated through analysis of the previous lot (where available). Ampoule-to-ampoule consistency, or lot homogeneity, ensures consistency in recovery and is demonstrated through analysis of ampoules pulled from across the lot. Lack of variability is demonstrated through the tight %RSD reported for the analysis. Acceptance criteria incorporates variability of the analysis.
- **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".
  Manufacture of Cerilliant standards is fully documented in a detailed batch record capturing all equipment utilized providing traceability to equipment calibration records. Calibration verification weigh tapes are included in each batch record.
- **Solution Standard Assay**
  Shows method used to assay solution to an independently prepared calibration solution and calibration solution qualification data
- **Neat Material Characterization Summary and Purity Factor**
  Cerilliant Certified Solution Standards begin with full characterization of the neat material including chromatographic purity and analyses for residual content including: water, solvent, and inorganic content.
  This approach ensures accuracy of the standard and its suitability for demanding quantitative applications.
  Table shows results of all analyses performed and calculated Purity Factor.
- **Chromatographic Purity**
  Cerilliant utilizes multiple techniques to determine chromatographic purity. Results must agree within 0.5% of each other. The Primary purity method is used for purity factor calculations. The Secondary purity method is utilized as a confirming method.
  This approach ensures proper resolution of impurities and related substances and protects against random analytical error that could result in improper purity assignment.
- **Purity Factor**
  Purity Factor – often referred to as "potency" is used to calculate the amount of material needed to achieve accurate concentration of the solution standard.
- **Identity**
  Cerilliant utilizes multiple methods to determine neat material identity. Each result must be consistent with compound structure. This approach protects against random analytical error that could result in improper identification.

CERTIFICATION & ASSESSMENT OF UNCERTAINTY

Proper certification should include assessment of uncertainty of the reference preparation

Neat Material Purity

- Uncertainty associated with all testing performed for neat material certification must be included.
- Chromatographic purity – Residual water – Residual solvent – Residual inorganic content
- Uncertainty influenced by sample size, instrument type, and analytical technique (number and type)

Mass Measurement

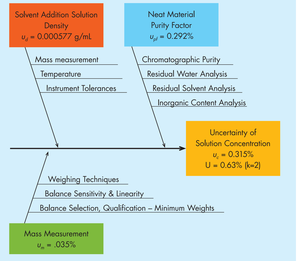
- Uncertainty associated with all weighing operations during standard production.
- Specific to the weighing technique, equipment used, scale of production, environment, and weighing procedures

Solvent Addition / Solution Density

- Uncertainty associated with the method of solvent addition.
- Consider Solvent temperature, glassware or balance tolerances, solvent density

Each of these processes was examined in detail and uncertainty determined using a combination of experimental results and instrument and process tolerances.

Cerilliant Model of Factors Impacting Solution Standard Uncertainty



Assessment of Uncertainty is a requirement for compliance with ISO 17025

UNCERTAINTY STATEMENTS

Uncertainty statements on a vendor's Certificate of Analysis are important for conveying the factors which impact uncertainty of the solution standard or neat reference material

Cerilliant Certificate of Analysis, Solution Standard

"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". All data for Purity Factor is provided in the Certificate of Analysis.

Vendor Certificate of Analysis Example 1, Solution Standard

"Uncertainties determined using repeatability and reproducibility data for balances and glassware from measurement systems analysis methodology, balance and glassware tolerances, raw material purity, and, where applicable, eccentricity and linearity values from an accredited calibration laboratory."

Vendor Certificate of Analysis Example 2, Neat Reference Material

"The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials."

Vendor Certificate of Analysis Example 3, Solution Standard

"The results are expressed as the certified value ± the expanded uncertainty. Certified values are unweighted means of concentrations determined by gravimetric preparation and chromatographic and titrimetric measurements [1]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95% confidence), calculated by combining a between-source variance incorporating inter-method bias with a pooled within-source variance following the ISO/… Guides [2]. The uncertainty includes both correction for estimated purity and allowance for differences among the concentrations determined by gravimetric preparation and chromatographic and titrimetric measurements."

Vendor Certificate of Analysis Example 4, Neat Reference Material

"The uncertainty in the certified property value is expressed as an expanded uncertainty, U, at a 95% confidence interval and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = k\mu_c$, where the coverage factor, k = 3.2 and μ_c is the combined uncertainty."

Vendor Certificate of Analysis Example 5, Neat Reference Material

"Standard Uncertainty: uncertainty values in this document are combined standard uncertainties expressed as Standard Uncertainty corresponding to one standard deviation. The components of Standard Uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components of stability are generally considered to be negligible unless otherwise indicated by stability studies."

Vendor Certificate of Analysis Example 6, Neat Reference Material

"This certified reference material (CRM) is produced and certified in accordance with ISO/IEC 17025 and ISO Guide 34. This CRM is traceable to SI unit kg and measured against NIST SRM. Certified content including uncertainty and expiry date are given on label."

CONSIDERATIONS AND CONCLUSIONS

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?
- Are 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 for those of the manufacturer alone or have they been experimentally verified for the manufacturing process?
- If the standard was diluted from a stock solution, does the uncertainty include uncertainty of the secondary dilution?

CONCLUSIONS

Results are only as accurate as the reference! Accuracy depends on robustness of the analysis and quality of the reference.

- In addition to chromatographic purity, full characterization of residual content including water, solvent, and inorganics are necessary for accurate solution preparation.
- Hygroscopicity and other changes in the neat material over time affect the purity factor of the neat material which ultimately impact accuracy of the solution standard concentration.
- Balance environment, weighing technique, and size of the weighing can significantly influence reference accuracy.
- Uncertainty determination should address all aspects of the production process and is a requirement for compliance with ISO 17025.
- Uncertainty statements on a vendor's Certificate of Analysis are important for conveying the factors which impact uncertainty of the reference standard.