Certified Solution Standards for Clinical Applications

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Introduction

Accuracy and reliability of clinical results and medical device performance is dependent on accuracy and reliability of the method of analysis, accuracy in the preparation of samples, and accuracy of the calibrators used.

Highly pure, well-characterized, solution based standards or reagents are a good and efficient alternative to the use of neat materials in clinical, toxicology and therapeutic drug monitoring applications.

Certified Solution Standards and Reagents offer a significant advantage over neat reference materials in terms of accuracy, consistency and stability.

Long term stability of solution based materials is achievable when appropriate parameters are chosen in the design, preparation, packaging, and storage.

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

What makes a Good Reference Standard - One Suitable for Quantitative Applications?

- Thoroughly & accurately characterized components
- Prepared using accurate, calibrated, and qualified pipettes, glassware & balances
- Traceability of all components
- High purity diluents and/or stabilizers, compatible with the compound(s)
- Analyzed to verify accuracy & consistency
- Uncertainty assessed and reported

High Quality Certified Ampouled Solutions and Spiking Solutions meet all these criteria and when properly designed, packaged, and stored, can remain stable for long periods of time enhancing laboratory productivity and efficiency

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Reference Standards Are Critical to the Quantitation of Drugs in a Clinical Setting

Different Approaches to Reference Standards

Certified Neat Reference Standard

- Analysts weigh neat materials at the bench to prepare volumetric solutions for use as stocks, calibrators and controls



Ampouled Certified Solutions

- Analysts use as-is or dilute volumetrically to stocks, calibrators and/or controls
- Widely used in clinical, forensic, toxicology, pharmaceutical and environmental testing



A Comparison of Approaches

	Ampouled Certified Solutions	Lab prepared (solutions from neat materials)
Stability over time	Years	Weeks-months
Lot to lot consistency / reproducibility	Large batches: large weighings, one lot available for an extended time & across locations	Frequent smaller weighings, multiple lots, repeat qualification
Homogeneity / concentration	Ampoule to ampoule and across the lot	Cannot be ensured – precipitation/evaporation Hygroscopicity of the neat can affect concentration from weighing to weighing
Efficiency	Reduced labor for bench preparation and certification Controlled substances can be exempt in solution	Repeated weighing, handling, qualification Handling of neat controlled substances requires additional documentation
Material usage/cost	Eliminates waste – stable single use format	More frequent preparation – more disposal
Contamination risk	Single use format – very low risk	Multiple use format – higher risk for bulk contamination
Convenience of use	Snap-N-Shoot®/Snap-N-Spike™	Weigh, dilute, qualify
Unstable/labile materials	Not suitable	Best prepared fresh

Accuracy, Consistency, & Stability achieved through proper Design & Preparation

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- Neat material characterization
- Solvent/diluent compatibility
- Accuracy of weighing operations
- Accuracy of solvent addition
- Packaging & storage
- Assessment of shelf life

Neat Material Characterization

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

- Residual Water & Hygroscopicity
  - A neat reference material may contain residual water and/or absorb moisture over time despite high chromatographic purity.
  - Residual water must be included in the purity factor for quantitative applications.
  - 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 must be included in the purity factor for quantitative applications.
  - 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.
  - As with residual solvent or water, trace inorganics must be included in the purity factor for quantitative applications.

Impact of Residual Water/Hygroscopicity

Changes in residual water content over time can significantly impact the purity factor

Compound	First Analysis Date	Second Analysis Date	First Analysis Water (%)	Second Analysis Water (%)	Months Stored Between Analyses	Increase in Water Content
Morphine	10/2007	5/2009	0.66	3.36	19	409%
Morphine-3-ß-D-Glucuronide	1/2007	4/2009	3.11	7.23	28	132%
Desmethyldoxepin	11/2007	4/2009	0.57	4.11	18	621%
Norhydrocodone HCl	6/2008	6/2009	1.25	3.12	12	150%
3'-Hydroxystanozolol-D_3	3/2008	6/2009	1.74	3.85	15	121%

Materials were stored under normal freezer conditions in sealed, screw-cap amber vials.
Water content was analyzed by Karl Fisher Coulometry based on USP method <921>.

Impact of Residual Content

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 5/2009	99.8	None Detected	< 0.1%	3.36	96.45
Morphine-3-ß-D-Glucuronide 1/2007	99.6	1.38	< 0.1%	3.11	95.10
Morphine-3-ß-D-Glucuronide 4/2009	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

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Larger weighings more accurate

- Improper balance selection can lead to high levels of uncertainty
- Minimum weighings should be established to achieve minimum relative error.
- Cerilliant specifies minimum weighings to achieve USP tolerances of ≤0.1% relative error.

Importtance 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%

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

Balance environment & weighing technique can significantly influence reference accuracy

- 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.

Solvent Addition

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Gravimetric addition of solvent provides reproducibility

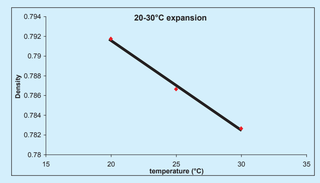
- Target solvent weight calculated from target volume by adjusting for density. Actual solvent weight can be calculated back into volume to report concentration in mg/mL
- Balance is more accurate than volumetric flask
- Temperature affects density thus affecting volume
- Eliminates subjectivity of visual fill line
- Weigh tapes provide traceability to SI units

Method	Batch Size		
	10mL	100mL	1000 mL
Volumetric flask standard error Source: ASTM E288-03, Standard specification for laboratory glassware, 2003	0.20%	0.08%	0.03%
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

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, 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

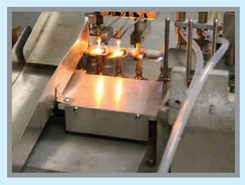
Solvent compatibility is critical to long term stability

- Solubility
  - Does the target compound dissolve at the required concentration?
  - Precipitation can occur over time or at reduced storage temperatures
- Compatibility with analysis
  - Solvent interferences in the chromatogram: UV cut-off; baseline effects
  - Non-polar solvents not ideal with reverse phase HPLC
  - Water not compatible with GC
- Solvent stability
  - THF/ethers form peroxides
- Compound stability in the solvent

Dispensing & Packaging

Snap-N-Shoot® and Snap-N-Spike™ Format Advantage vs. Solutions Stored in Volumetric Flasks

- Solution standards dispensed into single use volumes and flame sealed under inert atmosphere
- 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 and contamination, and promotes stability

- Expiration (shelf life) is established through real-time stability studies
- Solution purity and concentration are re-evaluated at multiple intervals
- Solutions properly designed and prepared can be stable for years

Compound/Solvent	Age of Stability Sample	Solution Purity		Analyzed Concentration	
		Original	Stability Interval	Original	Stability Interval
Fentanyl/methanol (ug/mL)	5 years	99.1%	99.9%	97.6	98.6
6-Acetylmorphine/ acetonitrile (ug/mL)	5.5 years	98.0%	99.5%	98.8	97.8
Nortriptyline HCl/ methanol (mg/mL)	5 years	99.8%	99.9%	0.995	0.970
Codeine/methanol (mg/mL)	5.5 years	99.9%	99.4%	0.989	0.995
Haloperidol/methanol (mg/mL)	6 years	99.8%	99.8%	0.988	0.970

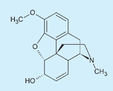
Concentration acceptance criteria for each of the examples = ± 3% and incorporates variability of the analysis.

Codeine

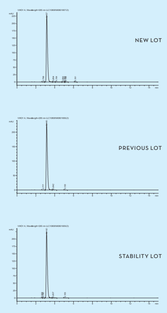
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Stability established at 5.5 years

Catalog Product: C-006, 1 mg/ml in methanol
Analysis Method: HPLC/UV
Column: Betasil Phenyl 4.6 x 150 mm
Mobile Phase: Acetonitrile::0.01M Phosphate Buffer (70::30)
Flow Rate: 0.8 mL/min
Wavelength: 285 nm
Calibration Curve: Linear Regression
Number of Points: 4
Linearity (r): 0.999



Solution	Lot Number	Manufacture Date	% Conc. Diff from New Prep	Solution Purity
New Lot	FE072108-01	7 / 2008	-	99.4%
Previous Lot	35012-94D	8 / 2006	2.4	99.5%
Stability Lot	35053-15B	1 / 2003	-0.7	99.4%



Fentanyl

Stability established at 5 years

Catalog Product: F-002, 100 µg/mL in methanol
Analysis Method: HPLC/UV
Column: Betasil Phenyl 4.6 x 150 mm
Mobile Phase: Acetonitrile::0.01M Phosphate Buffer (70::30)
Flow Rate: 1.0 mL/min
Wavelength: 220 nm
Calibration Curve: Linear Regression
Number of Points: 4
Linearity (r): 0.999



Solution	Lot Number	Manufacture Date	% Conc. Diff from New Prep	Solution Purity
New Lot	FE022508-02	2 / 2008	-	99.8%
Previous Lot	35315-35B	3 / 2006	-0.5	99.8%
Stability Lot	29875-71H	1 / 2003	-1.3	99.9%



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

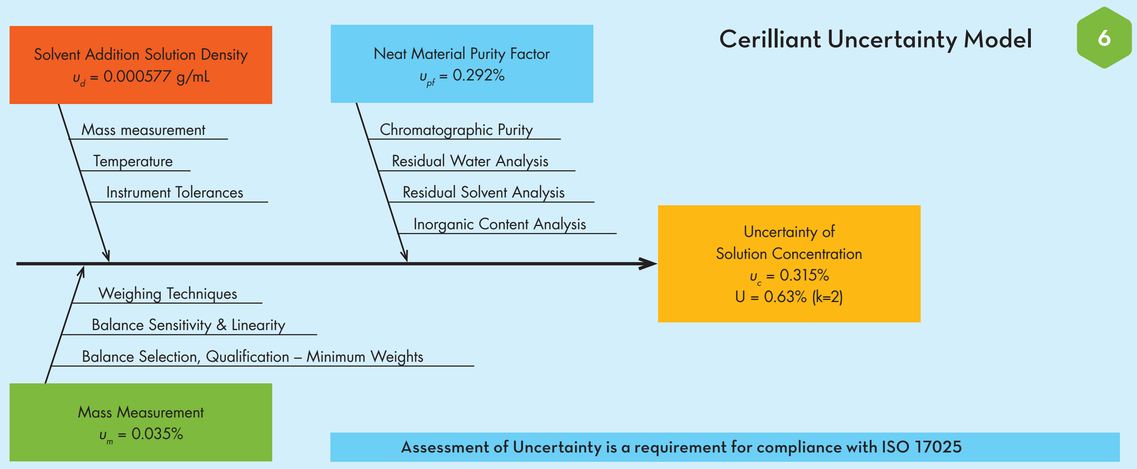
Solvent Addition

- 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 Uncertainty Model

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Assessment of Uncertainty is a requirement for compliance with ISO 17025

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Conclusion

Properly Prepared Certified Spiking Solutions™ & Solution Standards Are An Excellent Alternative to the Use of Neat Materials for Clinical and Toxicology Applications

- Single use format produced in large lots
  - Low risk of contamination
  - More efficient use of material
  - Improved consistency and accuracy
    - Larger weighings
    - Single lot used over longer periods of time and across locations
- Reduces labor and time for routine reference preparation at the bench
- Sealed containers and inert environment protect against evaporation and degradation
- Solution stability established through testing
- Uncertainty and traceability established
- USDEA exemptions for solutions of controlled substances available

Ampouled Certified Spiking Solutions & Solution Standards are Accurate, Consistent and Efficient