# 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

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

Different Approaches to Reference Standards

**Certified Neat Reference Standard** 

and environmental testing

• 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

High Quality Certified Ampouled Solutions and Spiking Solutions

# Weighing Accuracy

### Larger weighings more accurate

<ul> <li>Improper balance selection can lead to high levels of uncertainty</li> <li>Minimum weighings should be established to achieve minimum relative error.</li> <li>Cerilliant specifies minimum weighings to achieve USP tolerances of ≤0.1% relative error.</li> </ul>	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 ma	0.080%	0.45%		

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			

1000 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

0.045%

0.080%

0.0080%

• 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**

#### 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

Batch Size Method 10mL 100mL 1000 mL

#### Traceability of all components

- High purity diluents and/or stabilizers, compatible with the compound(s)
- Analyzed to verify accuracy & consistency
- Uncertainty assessed and reported

# A Comparison of Approaches

	Ampouled Certified Solutions	Lab prepared (solutions from neat materials)
Stability over time	Years	Weeks-months
Lot to lot consistency / reproducibility Lot to lot consistency / reproducibility Lot to lot consistency / reproducibility Large batches: large weighings, one lot available an extended time & across locations		Frequent smaller weighings, multiple lots, repeat qualification
Homogeneity / concentration	concentration Ampoule to ampoule and across the lot	
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 <sup>®</sup> /Snap-N-Spike <sup>™</sup>	Weigh, dilute, qualify
Unstable/labile materials	Not suitable	Best prepared fresh

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

- Neat material characterization • Solvent/diluent compatibility
- Accuracy of solvent addition



Increase

in Water

Content

409%

• Accuracy of weighing operations

Impact of Residual Water/Hygroscopicity

• Packaging & storage • Assessment of shelf life

Second

Analysis

Water (%)

3.36

Months

Stored

Between

Analyses

19

## **Neat Material Characterization**

Complete & accurate characterization of neat material is essential to accuracy of the solution		Changes can sig
<ul> <li>Residual Water &amp; Hygroscopicity         <ul> <li>A neat reference material may contain residual water and/ or absorb moisture over time despite high chromatographic purity.</li> <li>Residual water must be included in the purity factor for</li> </ul> </li> </ul>	Compound	First Analysis Date
quantitative applications.	Morphine	10/2007

#### meet all these criteria and when properly designed, packaged, and stored, can remain stable for long periods of time enhancing laboratory productivity and efficiency

#### 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

Volumetric flask standard error Source: ASTM E288-03, Standard specification for laboratory glassware, 2003	0.20%	0.08%	0.03%
Analytical balance uncertainty Balance Type Typical values per Mettler Toledo	5 Place 0.001%	5 Place 0.0001%	1 Place 0.009%
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



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

#### 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

• Compound stability in the solvent

# **Dispensing & Packaging**

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

• Solution standards dispensed into single use volumes and flame sealed under inert atmosphere

# Codeine

# Stability established at 5.5 years

Catalog Product: C-006, 1 mg/ml in methanol Analysis Method: HPLC/UV Betasil Phenyl 4.6 x 150 mm Column: Mobile Phase: Acetonitrile::0.01M Phosphate Buffer (70::30) 0.8 mL/min Flow Rate: H<sub>3</sub>C<sup>--</sup> Wavelength: 285 nm

# NEW LOT

#### 0.794 <sub>T</sub> 20-30°C expansion 0.57% difference in 0.792 concentration when 0.79 prepared volumetrically - 0.788 ح **6** 0.786 at 20° vs. 25°C 0.784 -0.782 -0.78 + 25 temperature (°C) 30 15 20

Source: Handbook of Thermophysical and Thermochemical Data, CRC Press

- THF/ethers form peroxides

- Absorption of moisture over time will impact subsequen weighing of the material and must be re-evaluated prio 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.

nt or to	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%
h	3'-Hydroxystanozolol-D <sub>3</sub>	3/2008	6/2009	1.74	3.85	15	121%

Second

Analysis

Date

5/2009

in residual water content over time

First

Analysis

Water (%)

0.66

nificantly impact the purity factor

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

• 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	Solu	Solution		yzed
	Stability	Pu	rity	Concentration	
	Sample	Original	Stability	Original	Stability
			Interval		Interval
Fentanyl/methanol	5 years	99.1%	99.9%	97.6	98.6
(ug/mL)					
6-Acetylmorphine/	5.5 years	98.0%	99.5%	98.8	97.8
acetonitrile (ug/mL)					
Nortriptyline HCl/	5 years	99.8%	99.9%	0.995	0.970
methanol (mg/mL)					
Codeine/methanol	5.5 years	99.9%	99.4%	0.989	0.995
(mg/mL)					
Haloperidol/methanol	6 years	99.8%	99.8%	0.988	0.970
(mg/mL)					

Calibration Number of I Linearity (r):	Curve: Linear Re Points: 4 0.999	egression	н	O CH	3 3 1001 A Numbergh-SE or LOUISINGED 100 100 100 100 100 100 100 10
Solution	Lot Number	Manufacture Date	% Conc. Diff from New Prep	Solution Purity	
New Lot	FE072108- 01	7 / 2008	-	99.4%	VIGT A, Rowingth-Old on LC10830803 100.0
Previous Lot	35012-94D	8 / 2006	2.4	99.5%	STABILITY LOT
Stability Lot	35053-15B	1 / 2003	-0.7	99.4%	

# Fentanyl



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

Certification & Assessment of Un	certainty		Solvent Addition Solution Density	Negt Material Purity Easter	Cerilliant Uncertainty Model 6
Proper certification should include assessment of	of uncertainty of the reference preparation		$v_d = 0.000577 \text{ g/mL}$	$u_{pf} = 0.292\%$	
<ul> <li>Neat Material Purity</li> <li>Uncertainty associated with all testing performed for neat material certification must be included.</li> <li>Chromatographic purity – Residual water – Residual solvent – Residual inorganic content</li> <li>Uncertainty influenced by sample size, instrument</li> </ul>	<ul> <li>Mass Measurement</li> <li>Uncertainty associated with all weighing operations during standard production.</li> <li>Specific to the weighing technique, equipment used, scale of production, environment, and weighing procedures</li> </ul>	<ul> <li>Solvent Addition</li> <li>Solution density</li> <li>Uncertainty associated with the method of solvent addition.</li> <li>Consider solvent temperature, glassware or balance tolerances, solvent density</li> </ul>	Mass measurement Temperature Instrument Tolerances	Chromatographic Purity Residual Water Analysis Residual Solvent Analysis Inorganic Content Analysis	Uncertainty of Solution Concentration

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





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#### Conclusion

Properly Prepared Certified Spiking Solutions<sup>™</sup> & 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**