Certified Solution Standards and Reagents for Therapeutic Drug Monitoring Applications

CERTIFIED SOLUTION STANDARDS & REAGENTS FOR THERAPEUTIC DRUG MONITORING APPLICATIONS

1

Abstract

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. The accuracy, stability, and consistency of these materials is critical to ensure accuracy of results in the analytical laboratory, in clinical applications and medical device performance.

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.

This poster presents the development and design of Certified Solution Standards and Reagents providing examples of pre-made solutions that exhibit multi-year stability. Factors critical to the selection of the analytes, diluents, storage and stability are discussed. These include: raw material handling, characterization and potency, certification and qualification of solutions, and homogeneity and stability of the solution. Certified Reference Standard Solutions and Reagents prepared in a diluent that promotes stability and packaged under argon in flame sealed ampoules can be stable for many years.

Multiple examples are presented. Also presented is a comparison of certified ampouled solutions vs. neat reference materials.

Certified Solution Standards and Reagents ensure accurate, consistent and reliable results in clinical and toxicology applications and medical device performance.

REFERENCE STANDARDS ARE CRITICAL TO THE QUANTITATION OF THERAPEUTIC DRUGS IN A CLINICAL SETTING

2

Results are only as accurate as the reference!

Accurate quantitative results depend on

- The purity of the reference material
- The accuracy in the preparation of the solution used in the analysis

TYPES OF REFERENCE STANDARDS AND HOW THEY ARE USED

Certified neat reference standard

- Analysts prepare volumetric solutions by weighing the neat materials and diluting just prior to use
- This may be a stock solution, calibration curve or control

Ampouled Certified Solutions

- Analysts use as-is or dilute volumetrically to stocks, calibrators and/or controls
- Ampouled solutions are widely used in the clinical, forensic and environmental testing industries

CHARACTERISTICS OF GOOD REFERENCE STANDARDS

3

- Thoroughly characterized components
- Use of accurate, calibrated pipettes & balances
- Analyzed to verify accuracy & consistency
- Traceability of all components
- High purity diluents and/or stabilizers, compatible with the compound(s)

High quality Ampouled Certified Solution Standards = Good Reference Standards

AMPOULED CERTIFIED SOLUTIONS – A BETTER ALTERNATIVE IN MOST CASES

4

	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 and 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
Convenience of use	"Snap (dilute) and Shoot"	Weigh, dilute, qualify
Efficiency	Reduced labor – eliminates 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
Unstable / labile materials	Not suitable	Best prepared fresh

PREPARATION OF CERTIFIED SOLUTIONS

5

NEAT MATERIAL

Complete characterization of the neat material is critical to the accuracy of the solution

Certification should include:

- Purity and impurities
  - Residual water, solvent, inorganic content (KF, headspace, micro-ash/ROI)
  - Chromatographic purity, resolution of impurities (LC, GC)
- Quantitation by assay (LC, GC, titration)
- Verification of identity (NMR, FTIR, MS)

Considerations

- Are vendor certified values complete, accurate and reliable?
- Reliability/repeatability of method?
- Is the compound more stable as salt vs. free base?
- Is the compound stable in the diluent?
- Is there an adjustment for salt form?
- Does the vendor provide uncertainty on the purity factor (potency)?

PREPARATION OF CERTIFIED SOLUTIONS

6

PURITY FACTOR (POTENCY) - SOURCES OF UNCERTAINTY

- Chromatographic purity – homogeneity of the neat and analytical uncertainty
- Residual solvent – sample preparation, weighing, and analytical response
- Residual water – sample weighing, influence of ambient moisture, analyst technique, and instrument tolerances
- Inorganic content – sample weighing and homogeneity of the sample

Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100]

- Assay value – reference standard uncertainty, sample preparation, and analytical uncertainty

PREPARATION OF CERTIFIED SOLUTIONS

7

DILUENT/SOLVENT CONSIDERATIONS

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

- Protic solvents – cocaine degrades in methanol over time but is stable in acetonitrile long term
- Oxazepam decomposes over time in methanol

Purity, identity and traceability

PREPARATION OF CERTIFIED SOLUTIONS

8

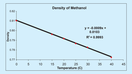
SOLUTION PREPARATION

Neat Material Handling

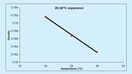
- Air or moisture sensitive compounds handled in inert atmosphere
- Use appropriate PPE – toxic and highly labile compounds must be handled in a glove box

Neat Material Weighing

- Use a qualified balance appropriate for the amount to be weighed
  - Calibrated with NIST traceable weights
  - 5-place, 6-place, 7-place?
  - Qualified to <0.1% relative error per USP
- Use weighing techniques that minimize error
  - Tongs vs. gloved hands
  - Balance equilibration
  - Material acclimated to conditions
  - Control of atmospheric conditions (vibration, air movement, temperature)
- Contamination – weighing operations should be isolated from other operations
- Larger weighings are more accurate



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

GRAVIMETRIC APPROACH

Add solvent by weight

- 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

Weighing has lower error than volumetric flask

- Standard error for balance vs. flask
- Balance read-out vs. visual fill line

Weighing tapes provide traceability

Control of lot to lot consistency

- Control of temperature/density variable
- Subjectivity of volumetric preparation technique

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

PREPARATION OF CERTIFIED SOLUTIONS

9

BALANCE SELECTION AND WEIGHING TECHNIQUE ARE CRITICAL TO WEIGHING ACCURACY

- Balance selection: Balances should be qualified and assigned minimum weighings specific to installed conditions and lab specified weighing techniques
- Weighing techniques: Handling of samples during weighing can have significant impact on weighing accuracy and uncertainty
- Certified Solutions prepared in bulk with larger weighing have reduced weighing error compared to smaller weighings typical when neat materials are weighed at the bench

WEIGHING ACCURACY				
Balance	7-place	6-place	5-place	4-place
Balance Resolution	0.0001 mg	0.001 mg	0.01 mg	0.1 mg
Sample Mass	Expanded Uncertainty*			
1 mg	0.045%	0.16%	8.0%	45.0%
10 mg	0.0045%	0.016%	0.80%	4.5%
100 mg	0.00075%	0.0017%	0.080%	0.45%
1000 mg	0.00060%	0.00024%	0.0080%	0.045%
CerilliantMinWt.**	1 mg	3 mg	20 mg	125 mg

*Calculated using typical values reported by Mettler Toledo and following the methods outlined in the "ISO Guide to the Expression of Uncertainty in Measurement" and includes contributions from standard deviation, sensitivity, and linearity.

**Minimum weights calculated by Mettler during balance qualification to achieve USP specified minimum relative error of NMT 0.1%

PREPARATION OF CERTIFIED SOLUTIONS

10

SOLVENT ADDITION

Gravimetric is more accurate than volumetric addition

METHOD	BATCH SIZE		
	10 mL	100 mL	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 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%

TEMPERATURE VS. DENSITY

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

PREPARATION OF CERTIFIED SOLUTIONS

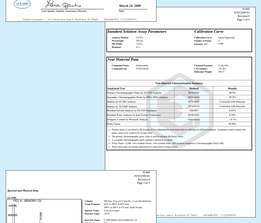
11

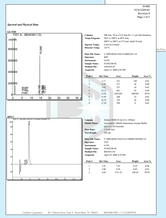
DISPENSING & PACKAGING

- Solution standards prepared in bulk can be dispensed into single use volumes and flame sealed under inert atmosphere
- Rigorous process controls ensure
  - Consistency of volume dispensed
  - Homogeneity from vial to vial and across the lot
  - No contamination
  - No degradation

CERTIFICATION OF THE SOLUTION STANDARD

12





Accuracy
Comparison to a primary source or certified second source – curve/calibration standard
Comparison of multiple independent preparations

Consistency
Lot-to-lot consistency verified by comparing to the previous lot

Homogeneity
Across the batch of ampoules/vials

Purity
Test for contamination and degradation

Traceability
– For all raw materials and calibrations

Stability
Established through comparison to older lots

SOLUTION STABILITY

13

Ampoulled solutions provide long-term stability

- Comparison to a primary source or certified second source
  Sealed amber ampoules protect from light and air
- Inert gas purge – displaces oxygen
- Silanization can reduce absorption to glass
- Sealed ampoules prevent concentration changes due to evaporation, absorption of moisture, and degradation
- Solution stability is assessed to determine shelf life
- Established by assay comparison to fresh solution
- Retest dates assigned to evaluate stability at set intervals
- Expiration dates established over time
- Sealed ampoulled solutions can exhibit excellent long term stability (>5 years for some)

SOLUTION STABILITY EXAMPLES

14

Stability Criteria

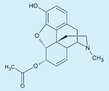
- No loss of chromatographic purity from originally established neat material value (within 0.5%)
- Concentration meets original acceptance criteria – reported differences may be reflective of method variability

COMPOUND/SOLVENT	AGE OF STABILITY SAMPLE	% DIFFERENCE IN CONCENTRATION BETWEEN STABILITY LOT AND NEW LOT
6-Acetylmorphine /acetonitrile	5.5 years	-2.3%
Nortriptyline HCl / methanol	5 years	-2.9%
Codeine / methanol	5.5 years	-0.7%
Haloperidol / methanol	6 years	-0.4%
Fentanyl /methanol	5 years	-1.3%

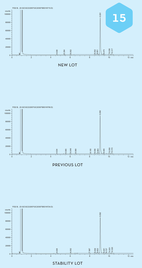
6-ACETYLMORPHINE

15

Catalog Product: ..... A-003, 100 µL in acetonitrile
Analysis Method: ..... GC/FID
Column: ..... DB-35ms 30 m x 0.53 mm ID, 1.0 µm film thickness
Temp program: ..... 60°C to 280°C at 40°C/min hold 7 min
Injector Temp: ..... Cool-on-Column
Detector Temp: ..... 325°C 230 nm
Calibration Curve: ..... Linear Regression
Number of Calibration Points: ... 3
Linearity (r): 0.995



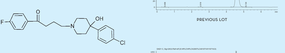
SOLUTION	LOT NUMBER	MANUFACTURE DATE	% CONC. DIFF FROM NEW PREP	SOLUTION PURITY
New Lot	FC022707-01A	3 / 2007	-	99.4%
Previous Lot	FC040405-02B	9 / 2005	2.0	99.2%
Stability Lot	34265-11B	7 / 2001	-2.3	99.5%



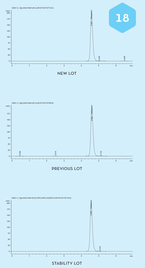
HALOPERIDOL

18

Catalog Product: ..... H-030, 1 mg/ml in methanol
Analysis Method: ..... HPLC/UV
Column: ..... Betasil Phenyl 4.6 x 150 mm
Mobile Phase: ..... Acetonitrile::0.02M Ammonium Acetate, pH=3 (60::40)
Flow Rate: ..... 1.0 mL/min
Wavelength: ..... 245 nm
Calibration Curve: ..... Linear Regression
Number of Points: 4
Linearity (r): 0.999



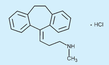
SOLUTION	LOT NUMBER	MANUFACTURE DATE	% CONC. DIFF FROM NEW PREP	SOLUTION PURITY
New Lot	29877-13G	1 / 2007	-	99.8%
Previous Lot	29877-13F	8 / 2005	0.3	99.8%
Stability Lot	29877-13B	11 / 2000	-0.4	99.8%



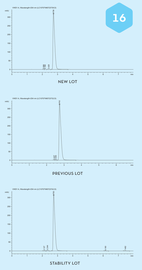
NORTRIPTYLINE HCl

16

Catalog Product: ..... N-907, 1 mg/mL in methanol
Analysis Method: ..... HPLC/UV
Column: ..... Betasil Phenyl 4.6 x 150 mm
Mobile Phase: ..... Acetonitrile::0.01M Phosphate Buffer (80::20)
Flow Rate: ..... 1.0 mL/min
Wavelength: ..... 254 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	FN071607-01	7 / 2007	-	99.9%
Previous Lot	35358-19A	7 / 2006	-0.7	99.9%
Stability Lot	35053-96A	5 / 2002	-2.9	99.9%

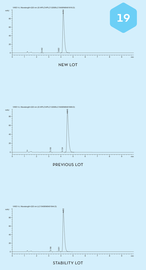


FENTANYL

19

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

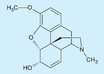
SOLUTION	LOT NUMBER	MANUFACTURE DATE	% CONC. DIFF FROM NEW PREP	SOLUTION PURITY
New Lot	FE022508-02	2 / 2008	-	99.8%
Previous Lot	35315-35B	3 / 2006	-0.5	99.8%
Stability Lot	29875-71H	1 / 2003	-1.3	99.9%



CODEINE

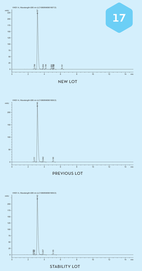
17

Catalog Product: ..... C-006, 1 mg/ml in methanol
Standard Analysis Method: ..... UV- Vis
Wavelength: ..... 285 nm
Slit Width: 1nm
Response: ..... 0.5 s
Calibration Curve: ..... Linear Regression
Number of Calibration Points: 4
Linearity (r): 0.999



Solution Purity Analysis Method: .... HPLC
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

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%



AMPOULED CERTIFIED SOLUTIONS ARE EFFICIENT, ACCURATE & CONSISTENT

20

- 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 standard preparation at the bench
- Sealed containers and inert environment protect against evaporation and degradation
- Solution stability established through testing
- DEA exemptions for solutions of controlled substances available

High quality Certified Solution Standards and Reagents are an excellent alternative to the use of neat materials for clinical and toxicology applications

Cerilliant®
science, smarter.®

AUTHORS

Isil Dilek PhD, Kevin Gates, Rebecca Johnson, Sherri Pogue, Mitzi Rettinger, Uma Sreenivasan PhD

© 2009 Cerilliant Corporation | 811 Paloma Drive | Round Rock, TX 78665