Custom Certified Stock Solutions for Use in Manufacture of IVD Reagents or Controls

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Abstract

Accuracy and quality of reagents are critical to medical device performance and patient care. IVD manufacturers often produce stock solutions for use in preparation of reagents. Many times concentration of the target analytes is very low, materials are difficult to handle, and may be unstable or toxic and require special handling. Concentration accuracy is dependent on extensive neat material characterization and precise weighing and dilution operations.

IVD manufacturers are looking to streamline operations but risks of outsourcing reagent preparation are high. One alternative approach is to outsource the preparation of stock solutions. Accurate shelf-stable certified stock solutions allow IVD manufacturers the ability to eliminate tedious operations and provide efficiency of resources while ensuring consistency, accuracy, and traceability of materials. Custom stocks solutions can be quantitatively prepared and certified eliminating material waste and non-conforming batches resulting from weighing errors or inaccurate material characterization allowing IVD manufacturers to focus on their core.



Accuracy and Quality of Reagents & Controls are Critical to Medical Device Performance and Patient Care

- High quality reagents / controls must be:
  - Accurate
  - Consistent from lot-to-lot
  - Shelf stable
- Issues in preparation include:
  - Concentration of the target analytes is very low
  - Materials may be difficult to handle, unstable or toxic requiring special handling
  - Analyte stability in matrix/diluent may be short term thus requiring multiple lot preparation in short amounts of time or lyophilization which creates another variable of uncertainty

As a result manufacturers frequently manufacture stock solutions

Stock/Spiking Solutions Options

Prepare from Neat Starting Material

- Volumetric solutions are prepared by weighing the neat materials, diluting, packaging, and assessing stability internally
- Requires personnel, procedures, equipment, QC/QA



Ampouled Certified Stock / Spiking Solutions

- Spiked directly into the reagent / control diluent
- Widely used in clinical, forensic, toxicology, pharmaceutical and environmental industries as spiking solutions



Why Consider Certified Stock / Spiking Solutions?

- Operational efficiency
- Limited staff resources
- Cost of valuable materials/waste
- Material handling issues
- Failed batches due to inconsistent raw materials
- OOT, OOS, and failed assays due to inconsistent stock preparation

Spiking Solution Critical Aspects of Preparation

Neat Material

- Sourcing – Internal or External
- Material Properties
- Certification

Manufacture

- Material Handling
- Diluent Selection
- Equipment Selection
- Gravimetric Preparation
- Dispensing Controls

Certification

- Purity
- Concentration
- Stability

Neat Materials - Certification

Identity

- Multiple techniques
  - 1D and 2D NMR
    - Proton
    - Carbon-13
    - Other nuclei
  - FTIR
  - GCMS, LCMS, LCMSMS
  - Other techniques as needed: EA, Optical Rotation, DSC, Melting Point, TGA
- Comparison to literature references

Purity / Potency

- Mass Balance – Orthogonal approach
  - Multiple techniques for chrom purity and residuals
  - Based on ISO Guide 34
  - Used by NIST
  - Appropriate mass balance equation critical

Purity and Impurities

Chromatographic Purity

- Purity and related substances
- Method development
  - Literature methods
  - Existing methods for similar compounds
  - Base line separation
  - Resolution of known impurities
- Use at least 2 techniques and different columns
  - values must agree within 0.5% of each other

Residual Impurities

- Residual water
  - USP <921>; system suitability
- Residual solvent by GC Headspace –
  - Cerilliant validated method or USP <467>
- Residual inorganic content
  - Micro ROI method based on USP <281> - less material with comparable results
- NMR evaluation
- EA or other techniques



Assignment of Purity Factor

Mass Balance Equation

- Incorporates chromatographic purity and related substances
- Assigned on an "as-is" basis – adjustments for salts made when preparing solution
- Equation may be modified to address impurities from orthogonal chromatographic techniques, chiral purity, etc.

$$PurityFactor = \left[[100 - (wt\%Solvents) - (wt\%H_2O) - (wt\%Inorganics)] * \frac{ChromPurity}{100} \right]$$

wt%Solvents: 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%Inorganics: 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

Complete Characterization Critical

Use of chromatographic purity alone can introduce significant error into the concentration of the spiking solution

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
Digoxin	95.5	0.10	< 0.1	0.56	94.91
Oxazepam Glucuronide	99.9	None Detected	2.37	8.96	88.58
Morphine	99.8	None Detected	< 0.1	3.36	96.45
Morphine-3-B-D-glucuronide 1/2007	99.6	1.38	< 0.1	3.11	95.10
Morphine-3-B-D-glucuronide 4/2009	99.6	1.38	< 0.1	7.23	91.00

Spiking Solution Preparation

Starting with well characterized neat material

Material Properties & Handling

- Compound stability at ambient conditions
- Hygroscopicity
- Potency/toxicity
- Sensitivity to air or light

Diluent Selection

- Solubility in desired diluent at desired concentration
- Stability considerations
- Suitability for end use



Spiking Solution Preparation

Solvent / Diluent Studies

- If not soluble in desired solvent or selected solvent impacts stability
- Various solvents
- Various concentrations

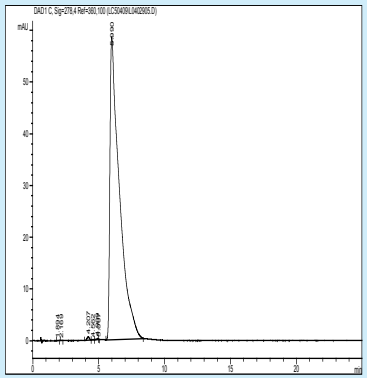
Stability Studies

- Various storage conditions evaluated
- Is purity consistent with neat?
- Precipitation?
- Accelerated to support shipping or lab use

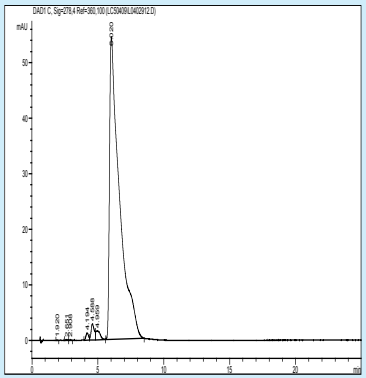
Diluent & concentration critical to long term stability

Diluent's Impact on Stability Example: Sirolimus

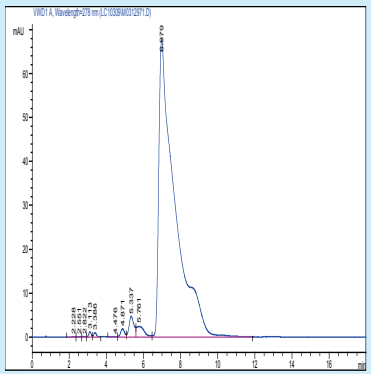
Rapid degradation in Methanol. Stable in Acetonitrile



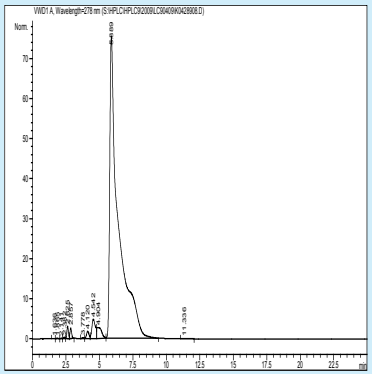
Freshly prepared solution
Time 0
Solution purity=99.6%



Ambient; 4 hours
Solution purity: 96.4%

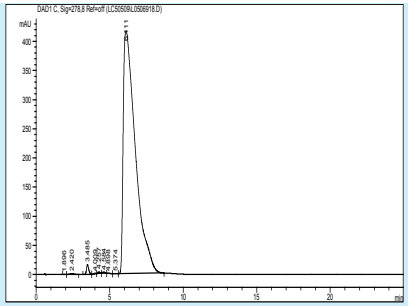


Freezer; 1 week
Solution purity: 95.0%

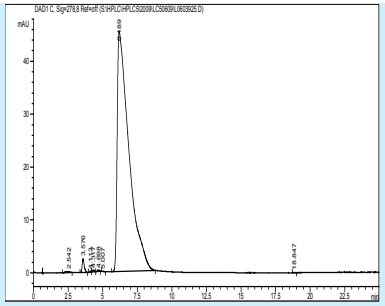


Freezer; 2 months
Solution purity: 93.5%

Methanol



Time 0
Solution Purity: 98.8%



Freezer; 4 weeks
Solution Purity: 98.5%

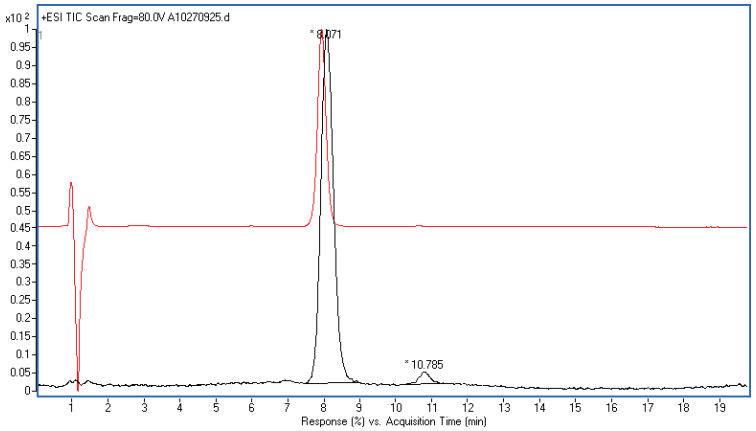
Stability Assessment: Purity of Sirolimus in Acetonitrile			
Testing Interval	Refrigerator	Freezer	Sub-freezer
Time 0	98.9		
1 Week	98.3	99.1	99.1
2 Weeks	96.6	98.7	98.2
4 Weeks	95.0	98.5	98.8
4 Months	-	-	99.0

- Stable during routine analysis under ambient conditions (3 hours).
- Degrades to 96% within 2 weeks in the refrigerator.

Acetonitrile

Evaluation of Solvent & Storage Conditions Example: 25-Hydroxyvitamin-D3

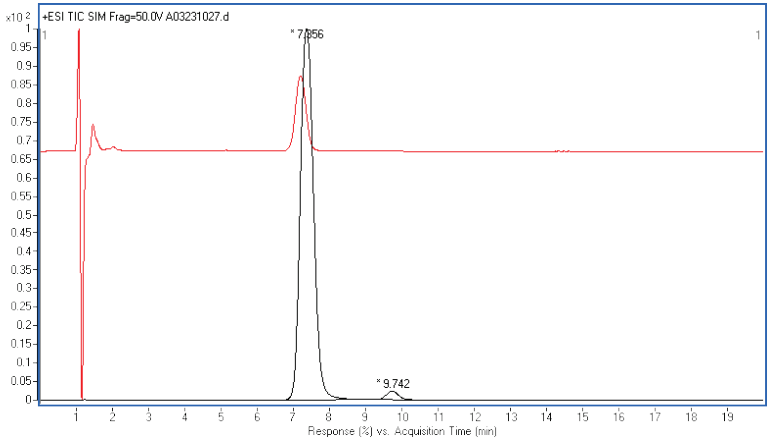
Acetonitrile



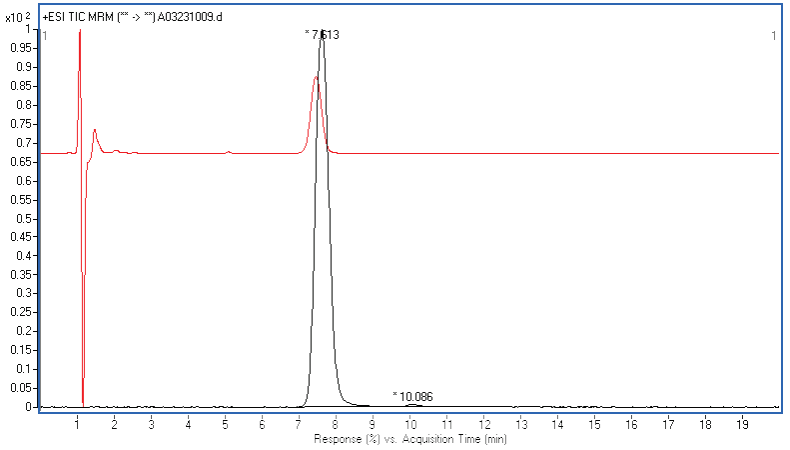
25-Hydroxyvitamin-D3, 100 ug/mL in acetonitrile. Freezer storage condition. Red is UV. Black is TIC.

HPLC and LCMS analysis of 25-Hydroxyvitamin-D3 in different solvents and storage conditions demonstrates improved performance of ethanol solution at sub-freezer conditions

Ethanol



25-Hydroxyvitamin-D3, 500 ng/mL in ethanol. Freezer 2 weeks.

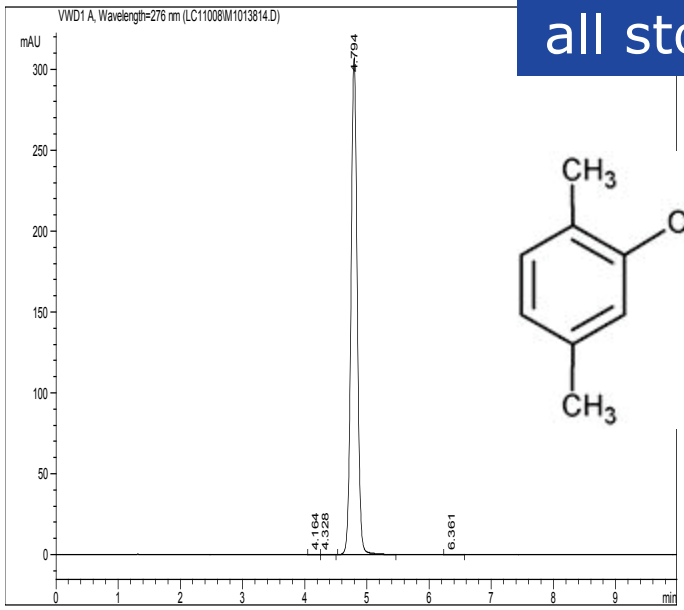


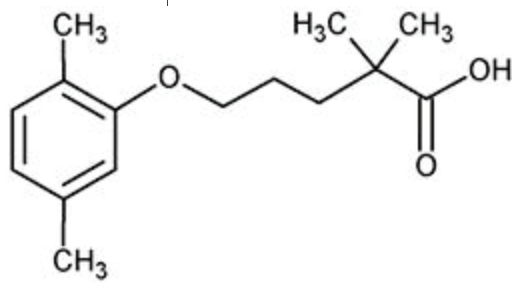
25-Hydroxyvitamin-D3, 500 ng/mL in ethanol. Sub-Freezer 2 weeks.

Accelerated Stability Example: Gemfibrozil

Storage Condition/ Test Interval	Gemfibrozil solution purity(%)
Initial (t=0)	99.9
Freezer (-1 to -25°C)	
1 week	99.9
2 weeks	99.9
4 weeks	99.9
Refrigerate (1 to 15°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

Stability exhibited at all storage conditions





Catalog Product: G-012, 1 mg/mL in methanol
Analysis Method: HPLC/UV
Column: Betasil Phenyl 4.6 x 150 mm
Mobile Phase: Acetonitrile::0.1% H_3PO_4 in Water
Flow Rate: 1.0 mL/min
Wavelength: 276 nm
Calibration Curve: Linear Regression
Number of Points: 3
Linearity (r): 1.000

Manufacturing Considerations

Materials

- Toxicity
- Hygroscopicity
- Sensitivity to air or light
- Static potential
- Viscosity / volatility

Equipment

- Balance selection for accurate weighing - 5,6 or 7 place
- Environmental controls – glove box
- Static bar
- UV filters
- Airline/respirators

Robust manufacturing practices critical to accuracy & consistency

Gravimetric Preparation

- Weight/Weight
- Higher precision vs. volumetric
- Balance selection
- Batch size flexibility vs. volumetric
- Traceability with weigh tapes
- Repeatability

Dispensing

- Equipment checks
- Line purge
- Tubing & syringes
- Sampling plans
- Segregation
- Evaporation control

Mass Measurement Considerations

Appropriate balance selection and qualification are critical to ensuring accuracy of the stock solution

- Improper balance selection can lead to high levels of uncertainty
- Wide range of qualified balances used
- Balances qualified in their installed state – calibrations semi-annually
- Minimum weighings established to achieve USP specified minimum relative error of NMT 0.1%.
- Calibration verification procedures – weekly & pre-use

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%

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

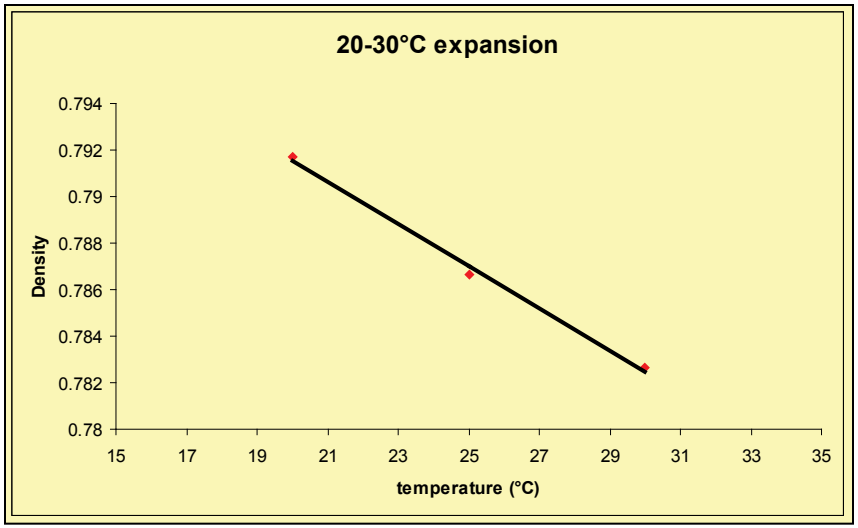
Weighing Technique can significantly influence accuracy

- Accuracy and repeatability of weighing can be influenced by:
  - tongs vs. gloved hands
  - balance equilibration time
  - sample and solvent temperature
  - ambient temperature
  - vibrations
  - movement of air
- Repeatability studies may identify flaws in processes

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.

Gravimetric Diluent 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



Density of Methanol 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
- Gravimetric addition provides traceability to SI units of mass
- Eliminates subjectivity of fill lines

Source: Handbook of Thermophysical and Thermochemical Data, CRC Press

Volumetric Preparation – more than you need?

Material wasted based on volume needed and available flask
Assuming a 5-place balance; Concentration 1 mg/mL

volume needed	volumentric flask sizes							
	25	50	100	200	250	500	1000	2000
5	20	45	95	195	245	495	995	1995
15	10	35	85	185	235	485	985	1985
25	0	25	75	175	225	475	975	1975
35		15	65	165	215	465	965	1965
45		5	55	155	205	455	955	1955
55			45	145	195	445	945	1945
65			35	135	185	435	935	1935
75			25	125	175	425	925	1925
85			15	115	165	415	915	1915
95			5	105	155	405	905	1905
120				80	130	380	880	1880
145				55	105	355	855	1855
170				30	80	330	830	1830
195				5	55	305	805	1805
245					5	255	755	1755
295						205	705	1705
345						155	655	1655
395						105	605	1605
445						55	555	1555
495						5	505	1505
595							405	1405
695							305	1305
795							205	1205
895							105	1105
995							5	1005
1095								905

Example:
Need 120 mL
Waste 80 mg

Typical minimum weighing on a 5-place balance is 25mg.
If using 4-place balance, typical minimum weighing = 125mg

- Considerations
  - Material costs
  - Waste disposal
  - Cost of remake
  - Use of resources

How much are you discarding?

Dispensing

Ampouled format and inert atmosphere protects from hygroscopicity, degradation, evaporation, & contamination - Promotes Stability

- Dispensed into ampoules - single use volumes
- Dispensing equipment selection dependent on batch size and material properties (viscosity, volatility)
- Batch homogeneity prior to dispensing ensured with thorough mixing - stirring or sonicating
- Material specific controls employed as needed: continuous chilling, continuous stirring, nitrogen blanket over bulk material
- Flame sealed under inert atmosphere



Certification of the Stock/Spiking Solution

Purity & Concentration are Analytically Verified

Consistency

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

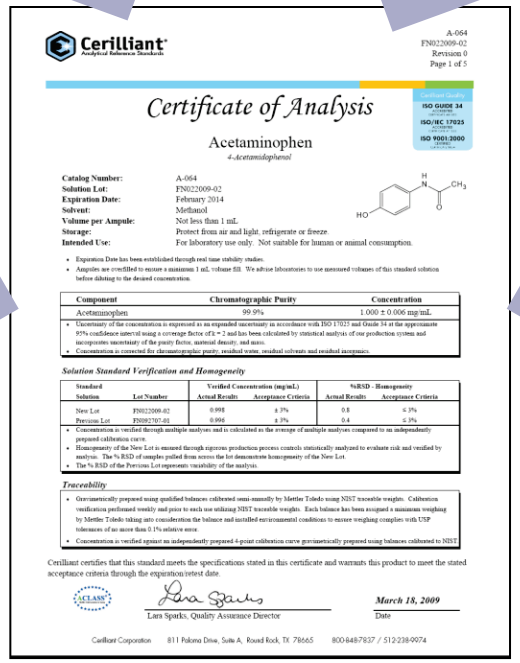
Homogeneity

Across the batch of ampoules/vials

Accuracy

Comparison to a primary source or certified second source – curve/calibration standard

Comparison of multiple independent preparations



Cerilliant

A-064
FN022009-02
Revision 0
Page 1 of 5

Certificate of Analysis

ISO GUIDE 34
ISO/IEC 17025
ISO 9001:2000

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, gravimetric 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	Acceptance Criteria	%RSD - Homogeneity Actual Results	Acceptance Criteria
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.

Lara Sparks, Quality Assurance Director — *March 18, 2009* Date

Cerilliant Corporation 811 Paloma Drive, Suite A, Round Rock, TX 78665 800-848-7837 / 512-238-9074

Purity

Consistent with neat material

No contamination or degradation

Stability Assessment

Solution purity and concentration re-evaluated at multiple intervals

- Real-time stability of older lots upon release of new lot – concentration comparison to calibration curve
- New product assigned retest date and tested each year until shelf life is established
  - Purity and concentration
- Accelerated stability if not assessed during development – determination of suitability for shipment under less controlled conditions
  - Shipping studies determined extremes encountered during transit



Stability Examples

Properly designed and prepared ampouled stock solutions can be stable for many years

Compound/Solvent	Age of Stability Sample	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.

A Comparison of Approaches

	Ampouled Certified Solutions	Internally prepared (solutions from neat materials)
Stability over time	Years	Weeks-months
Lot to lot consistency / reproducibility	Validated production process with established uncertainty; verification from lot-to-lot	Must establish and validate process
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	Streamlines operations and reduces labor for preparation & 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 format	More frequent preparation / weighings fixed to Class A volumetric quantities unless w/w – more disposal
Contamination risk	Single use format – very low risk	Multiple use format – higher risk for bulk contamination
Convenience of use	Snap-N-Spike®	Weigh, dilute, qualify

Ampouled Certified Spiking Solutions®
An Excellent Cost-Effective Alternative

- Eliminates tedious weighing operations
- Provides efficiency of resources while ensuring consistency, accuracy, and traceability of materials
- Eliminates material waste
- Improves scrap rate resulting from weighing errors or inaccurate material characterization
- Eliminates OOT, OOS, or failed assays due to inconsistency in solution preparation
- Custom stock solutions can be quantitatively prepared and certified providing additional convenience of use and minimizing internal handling

Allowing IVD manufacturers to focus on their core

Cerilliant®

Analytical Reference Standards



science, **smarter.**®

Cerilliant Quality

ISO GUIDE 34
CERTIFICATE AR1353

ISO/IEC 17025
CERTIFICATE AT1352

ISO 9001:2008
CERTIFICATE 3854