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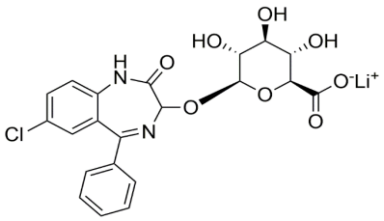
# Certified Reference Material - Certificate of Analysis

## Oxazepam glucuronide, Primary Measurement Standard

*7-Chloro-2,3-dihydro-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl-β-D-glucopyranosiduronic acid lithium salt*

**Product No.:** O-057-1ML
**Lot No.:** FN03222304
**Description of CRM:** Oxazepam glucuronide lithium salt in Methanol (Solution)
Nominal concentration, as free carboxylate, is adjusted for lithium salt content.
**Retest Date:** July 2025 See Stability Section
**Storage:** Store unopened in freezer (-10 °C to -25 °C).
**Shipping:** Ship cold. See Stability Section
**Chemical formula:** $C_{21}H_{18}ClLiN_2O_8$
**CAS No.:** NA
**Regulatory:** Canadian TK # 061-1861




| Analyte | Certified Concentration ± associated uncertainty $U$, $u = k * u$ ($k = 2$) |
|---|---|
| **Oxazepam glucuronide** | **100.0 ± 0.6 µg/mL** |

**Metrological traceability:** Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 3.

**Measurement method:** The certified value is calculated from high precision weighing of thoroughly characterized starting material. See "Details about certification process" on page 3.

**Intended use:** This Certified Reference Material is suitable for the in vitro identification, calibration, and quantification of the analyte(s) in analytical and R&D applications. Not suitable for human or animal consumption.

**Minimum sample size:** 1 µL for quantitative applications

**Instructions for handling and correct use:** Concentration is corrected for chromatographic purity, residual water, residual solvents, and residual inorganics. No adjustment required before use.
Users should quantitatively transfer desired volume using established good laboratory practices to spike into matrix or to dilute to the desired concentration. Each ampoule is intended for one-time use.
Nominal concentration, as free carboxylate, is adjusted for lithium salt content. No adjustment required before use.

**Health and safety information:** Danger. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

**Accreditation:** Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.

ANAB ANSI National Accreditation Board ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

James Schmitt, Quality Assurance Manager

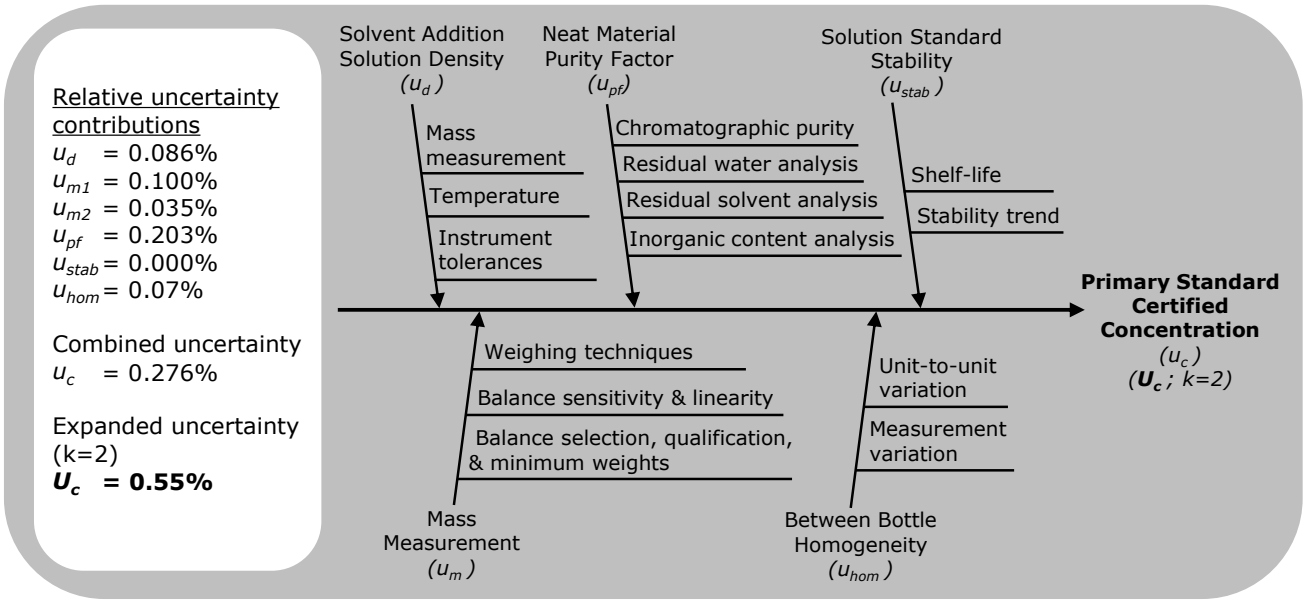
***July 16, 2024***
Issue Date

Cerilliant Corporation, 811 Paloma Drive, Suite A, Round Rock, TX, 78665, USA,
Tel: 800-848-7837 / 512-238-9974; www.cerilliant.com

| | |
|---|---|
| **Packaging:** | 2 mL amber USP Type 1 glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum of 1 mL volume can be transferred when using a 1mL Class A volumetric pipette. |
| **Details on starting materials:** | Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques and is assigned a Mass Balance Purity Factor. Spectral data is provided on subsequent pages of this CoA. |
| **Certificate of Origin:** | Cerilliant Corporation certifies no material of animal origin (BSE/TSE) was used in the preparation of this product. |
| **Country of Origin:** | United States of America |

**Associated uncertainty:**

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the mass balance purity factor, material density, balance, weighing technique, and homogeneity. Uncertainty components of the gravimetrically prepared Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Uncertainty contribution from neat material homogeneity was established to be negligible through establishment of process controls and verification of the control process. Stability uncertainty was determined to be negligible by regression analysis.



**Details on metrological traceability:**

- This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.10% relative error.
- Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- The density and material Mass Balance Purity Factor of each raw material is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations.

**Details about certification process:**

This standard has been prepared and certified under the ISO 17034, ISO/IEC 17025, and ISO 9001 standards. This standard meets the requirements of a Certified Reference Material and a Primary Standard as defined by ISO and is traceable to the SI and higher order standards through an unbroken chain of comparisons.

- Nominal concentration is calculated based on: the actual mass; Mass balance purity factor of the analyte(s); measured mass of the solution; and the density of the pure diluent at 20°C.
- Fill volume is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- Concentration is verified against an independently prepared calibration solution gravimetrically prepared.

- Additional certification information available upon request.

### *Solution Standard Verification*

*Concentration accuracy and within- and between-bottle homogeneity are analytically verified against an independently prepared calibration solution.*

| **Standard Solution Assay Parameters** | | **Calibration Curve** | |
|---|---|---|---|
| **Analysis Method:** | HPLC/UV | **Calibration Curve:** | Linear Regression |
| **Column:** | Ascentis Express F5, 2.7 µm, 3.0 x 100 mm | **Number of Points:** | 4 |
| **Mobile Phase:** | Acetonitrile:0.1% Phosphoric acid in Water (20:80) | **Linearity (r) :** | 1.000 |
| **Flow Rate:** | 2.0 mL/min | | |
| **Wavelength:** | 238 nm | | |

| | | **Verified Concentration (µg/mL)** | **%RSD - Homogeneity** |
|---|---|---|---|
| **Standard Solution** | **Lot Number** | **Actual Results** | **Actual Results** |
| New Lot | FN03222304 | 102.7 | 0.1 |

- Concentration is verified through multiple analyses and is calculated as the average of multiple analyses compared to an independently prepared calibration solution.
- Within-sample and between-sample homogeneity of the New Lot is ensured through rigorous production process controls statistically analyzed to evaluate risk and verified by analysis. Multiple samples pulled from across the lot using a random stratified sampling plan were analyzed to verify homogeneity. % RSD results shown above for the New Lot demonstrate ampoule-to-ampoule homogeneity.

### *Analyte Certification - Mass Balance Purity Factor*

*Each analyte is thoroughly identified and characterized using an orthogonal approach. A mass balance purity factor is assigned incorporating chromatographic purity and residual impurities. The mass balance purity factor and salt adjustment are utilized to calculate the weighing adjustment necessary to ensure accuracy of the solution standard concentration.*

| | | | |
|---|---|---|---|
| **Material Name:** | Oxazepam glucuronide lithium salt | **Molecular Weight (base):** | 461.83 |
| **Material Lot:** | FN02202306 | **Molecular Weight (salt):** | 468.77 |
| **Chemical Formula:** | $C_{21}H_{18}ClLiN_2O_8$ | **Salt Adjustment:** | 1.015 |
| **CAS Number:** | NA | | |

***Material Characterization Summary***

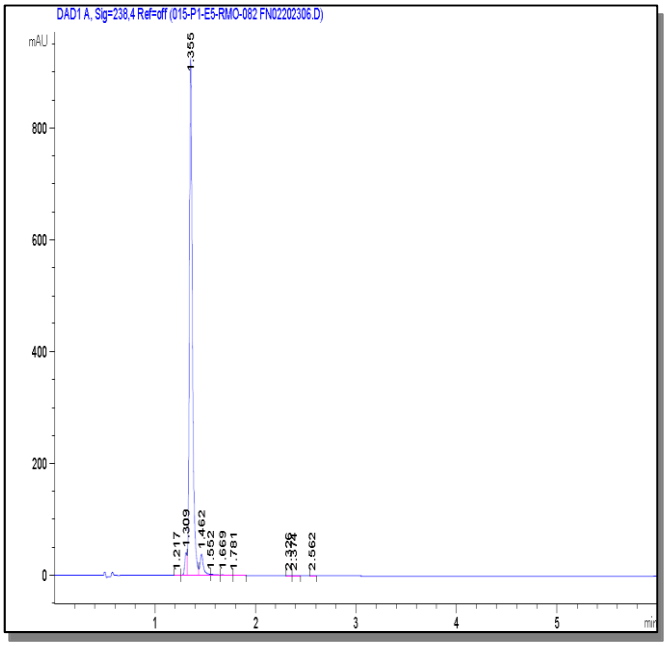
| Analytical Test | Method | Results |
|---|---|---|
| Chromatographic Purity by HPLC/UV Analysis | 20384348 | 99.5% [1,2] |
| Identity by LC/MS Analysis | 20384217 | Consistent with Structure |
| Identity by $^1$H-NMR Analysis | 20384224 | Consistent with Structure |
| Residual Solvent Analysis by GC/FID Headspace | 20397799 [3] | 0.08% |
| Residual Water Analysis by Karl Fischer Coulometry | 20384212 [3] | 4.64% |
| Ion chromatography (Cation Scan) | Outsourced | 1.35% Li |
| Mass Balance Purity Factor | | 94.83% |

[1] Purity is the sum of isomeric peaks
[2] 0.01% Oxazepam detected by HPLC/UV analysis
[3] Validated analytical method

- The chromatographic purity is calculated as the average of two independently performed analyses utilizing two different methods. Acceptance criteria requires the purity values to be within 0.5% of each other.
- The chromatographic purity value is used to calculate the Mass Balance Purity Factor.
- Mass Balance Purity Factor = [(100 - wt% residual solvent - wt% residual water - wt% residual inorganics) x Chromatographic Purity/100].
- Mass Balance Purity Factor does not include adjustment for chiral and/or isotopic purity.

## *Spectral and Physical Data*

### HPLC/UV




**Column:** Ascentis Express F5, 2.7 µm, 3.0 x 100 mm

**Mobile Phase:** A: Acetonitrile
B: 0.1% Phosphoric acid in Water

**Gradient:**

| Time (min) | % A | % B |
|---|---|---|
| 0.0 | 25 | 75 |
| 4.0 | 70 | 30 |
| 5.0 | 70 | 30 |
| 5.1 | 25 | 75 |
| 7.0 | 25 | 75 |

**Flow Rate:** 0.8 mL/min

**Wavelength:** 238 nm

**Sample Name:** FN02202306

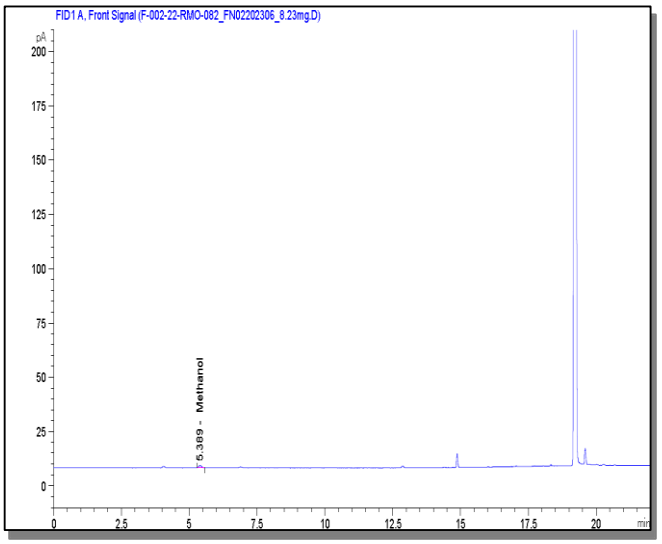
**Acquired:** June 15, 2023

| Peak # | Ret Time | Area % |
|---|---|---|
| 1 | 1.22 | 0.01 |
| 2 | 1.31 | 2.82 |
| 3 | 1.36 | 92.23 |
| 4 | 1.46 | 4.38 |
| 5 | 1.55 | 0.34 |
| 6 | 1.67 | 0.17 |
| 7 | 1.78 | 0.03 |
| 8 | 2.33 | 0.01 |
| 9 | 2.37 | 0.00 |
| 10 | 2.56 | 0.01 |

Purity is the sum of isomeric peaks 2, 3 and 4
Peak 8 is identified as Oxazepam

## *Spectral and Physical Data (cont.)*

### Residual Solvent Analysis by GC/FID Headspace

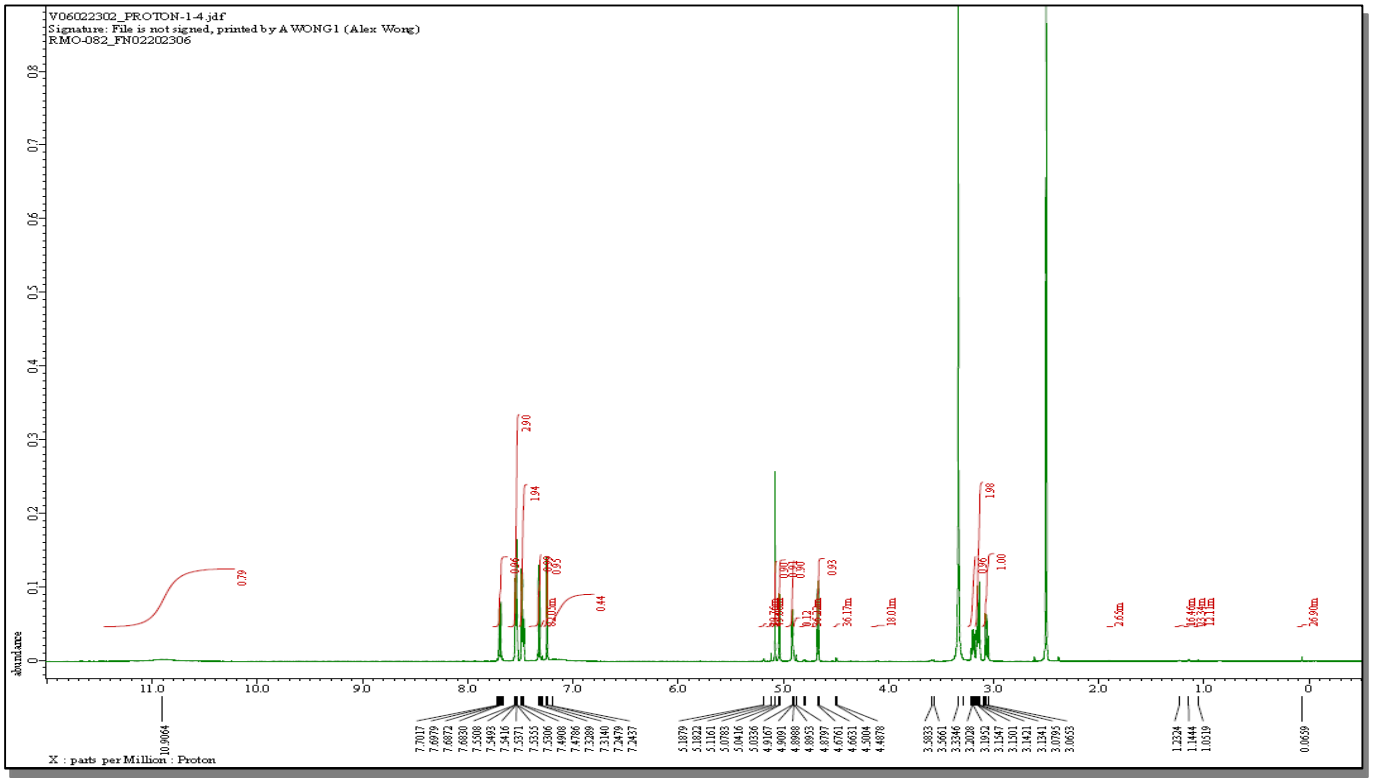



| | |
|---|---|
| **Column:** | DB-ALC1 30 m x 0.53 mm, 3 µm film thickness |
| **Temp Program:** | 40°C hold 12 min to 220°C at 40°C/min hold 5.5 min |
| **Carrier Gas:** | Helium |
| **Flow Rate:** | 2.0 mL/min |
| **Detector Heater Temp:** | 250°C |
| **Injector:** | Headspace Sampler |
| **HS Oven Temp:** | 60°C |
| **Vial Equilibration:** | 10 minutes |
| **Sample Name:** | FN02202306 |
| **Acquired:** | June 08, 2023 |

| Peak | Compound | Area | Weight % |
|---|---|---|---|
| 1 | Methanol | 8.23 | 0.08 |
| 2 | NMP | NA | NA |
| **Total** | | | **0.08** |

### $^{1}$H NMR

**Instrument:** JEOL ECZ600R
**Solvent:** DMSO-$D_6$



***Spectral and Physical Data (cont.)***

**LC/MS**

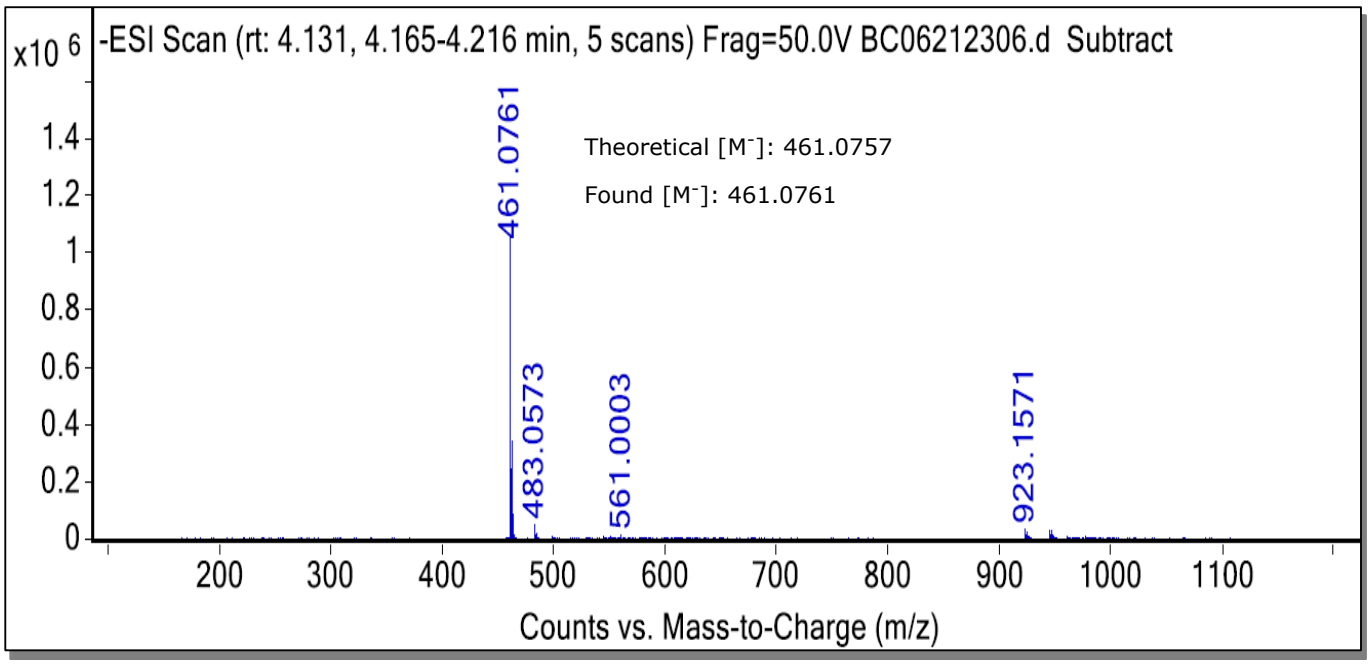
**Column:** Ascentis Express C18, 2.7 µm, 3.0 x 50 mm

**Mobile Phase:** A: 0.1% Formic acid in Water
B: Acetonitrile

**Gradient:**

| Time (min) | % A | % B |
|---|---|---|
| 0.0 | 90 | 10 |
| 0.5 | 90 | 10 |
| 4.0 | 50 | 50 |
| 5.8 | 50 | 50 |
| 6.0 | 90 | 10 |
| 8.0 | 90 | 10 |

**Flow Rate:** 0.4 mL/min
**Scan Range:** 115-1200 amu
**Ionization:** Electrospray, Negative Ion
**Instrument:** Agilent 6545XT QTOF
**Acquired:** June 21, 2023



***Stability***

*Short term stability studies have been performed in multiple storage conditions for a period of up to four weeks. Short term data is utilized to support transport conditions and normal laboratory use. Real-time stability studies are performed at the recommended storage conditions over the life of the product.*

***Short Term Stability:*** *A summary of stability findings for this product is listed below.*

| Storage Condition | Targeted Mean Kinetic Temperature (MKT) | Time Period/Result |
|---|---|---|
| Freezer | -20°C | No decrease in purity was noted after four weeks. |
| Refrigerator | 5°C | |
| Room Temperature | 20°C | 0.59% decrease in purity was noted after four weeks. |
| 40°C | 40°C | 1.31% decrease in purity was noted after three weeks. |

***Transport/Shipping:*** *Ship cold.*

***Long Term Stability:*** *Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 12 months has been established through real-time stability studies.*

***Commutability***

This standard is a solution of a pure substance in an organic solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

***COA Revision History***

| Revision No. | Date | Reason for Revision |
|---|---|---|
| 00 | August 24, 2023 | Initial version. |
| 01 | July 16, 2024 | Revised Retest Date of July 2024 to July 2025. |
| | | Revised Quality Assurance Manager signature. |
| | | Added Long Term Stability data. |
| | | |