

# Long-Term Stability of Novel Synthetic Opioids in Blood

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## INTRODUCTION

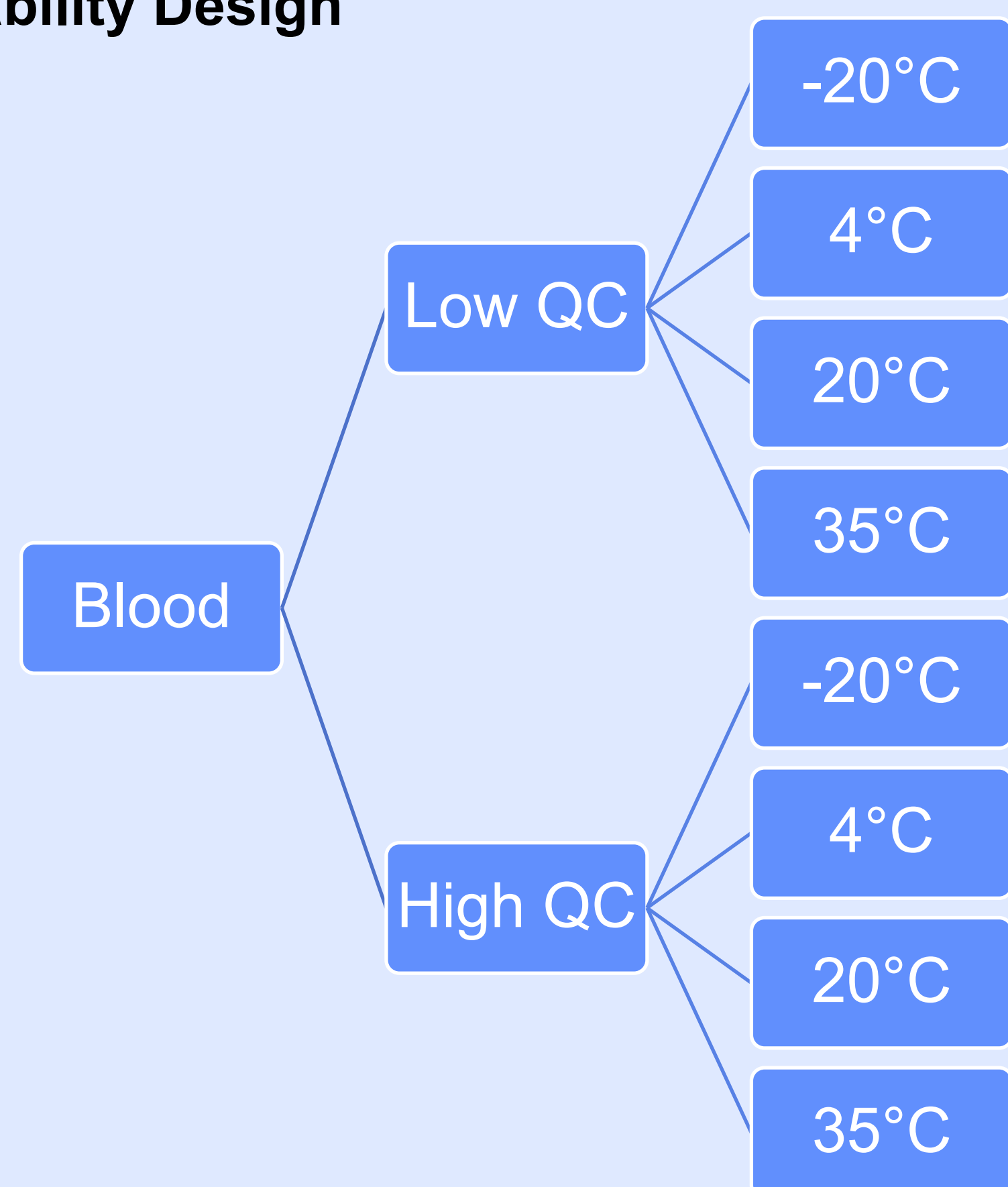
Recently, there has been an increase in overdose deaths due to novel synthetic opioids (NSO). Due to backlogs experienced by many forensic laboratories, samples containing these analytes could be stored for an unknown amount of time before analysis; therefore, it is important to understand their stability in a variety of storage conditions. The purpose of this research was to investigate the stability of AH-7921, U-47700, U-49900, U-50488, MT-45, W-15, and W-18 in blood at various temperatures over 36-weeks.

## MATERIALS AND METHODS

### Instrumentation

An Agilent 1290 Infinity II Liquid Chromatograph system equipped with an Agilent 6470 Triple Quadrupole Mass Spectrometer (Santa Clara, CA) was used for instrumental analysis. A Poroshell 120 EC-C18 column (100 mm x 3.0 mm x 2.7 μm) was utilized with a mobile phase consisting of 5 mM ammonium formate with 0.05% formic acid in water/0.1% formic acid in methanol. Separation was achieved using gradient elution at 0.5 mL/min.

### Stability Design



A total of 9 time points were analyzed in duplicate in this study (T<sub>0</sub>, 3 days, 2, 3, 4, 8, 12, 16, and 36 weeks). Samples were extracted and analyzed using a previously validated method [Lowry, *Forensic Toxicol*, 2019]. An additional protein precipitation step was added to reduce solvent usage and time. Stability was determined compared to T<sub>0</sub> and deemed acceptable if within ±20%.

## RESULTS AND DISCUSSION

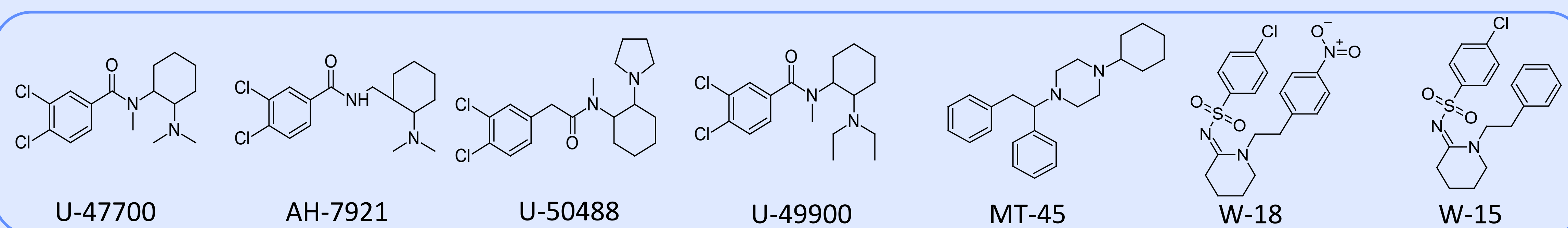


Figure 1. Structures of seven novel synthetic opioids

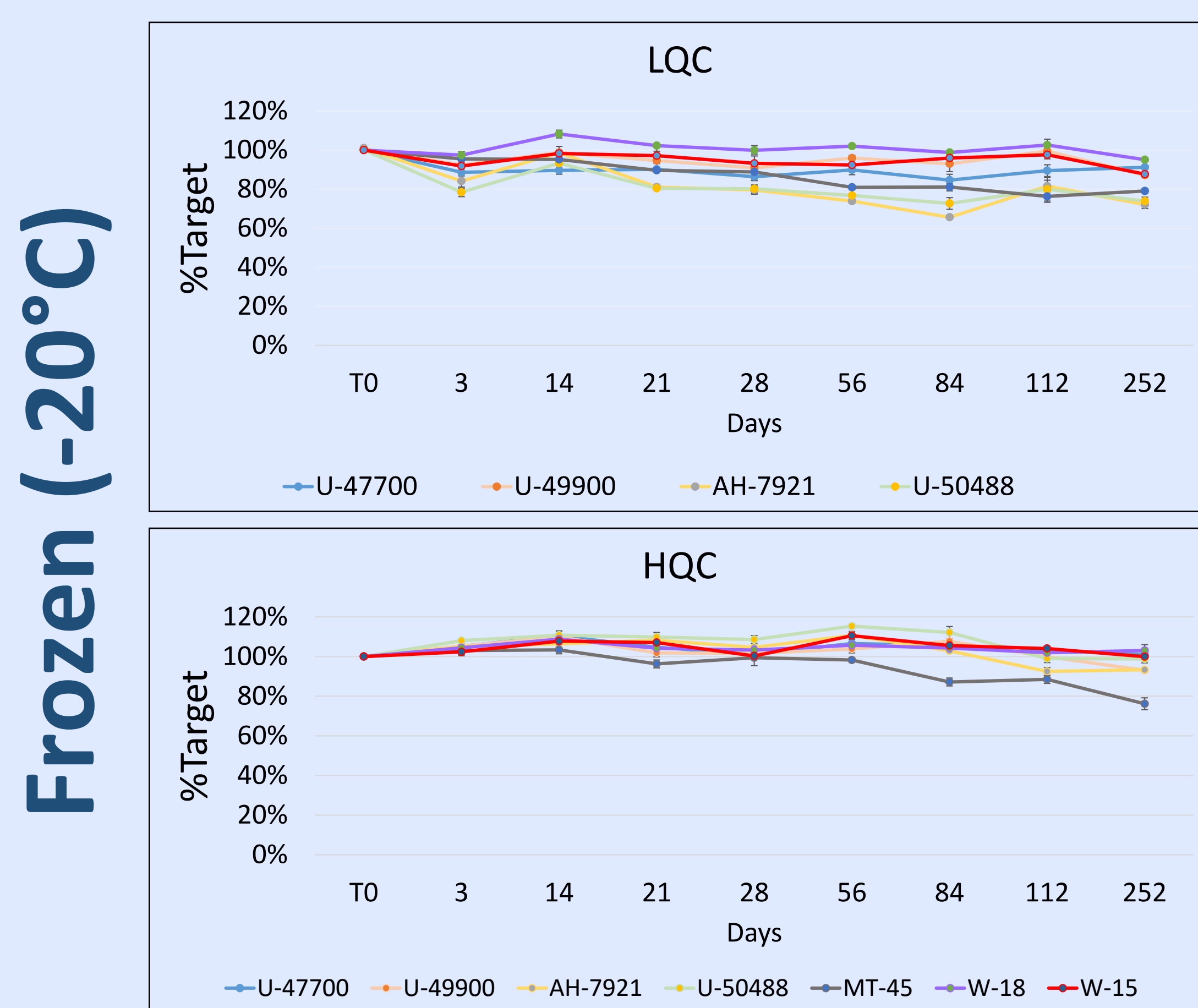


Figure 2. Frozen stability of U-47700, U-49900, AH-7921, U-50488, MT-45, W-18, and W-15 at a) 0.75 ng/mL (2.5 ng/mL for W-18) (LQC) and b) 80 ng/mL (HQC)

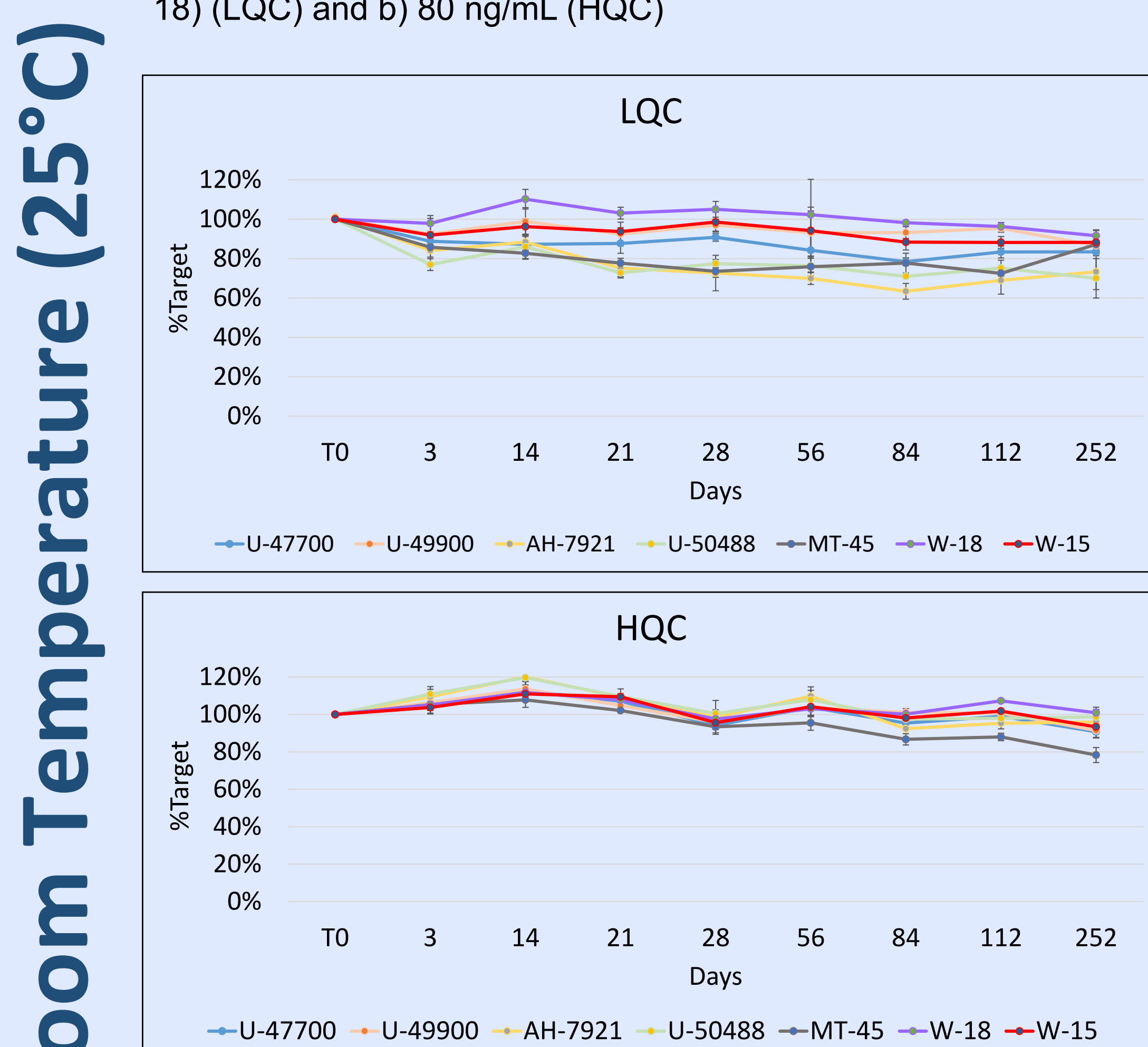


Figure 4. Room temperature stability of U-47700, U-49900, AH-7921, U-50488, MT-45, W-18, and W-15 at a) 0.75 ng/mL (2.5 ng/mL for W-18) (LQC) and b) 80 ng/mL (HQC)

### Refrigerated (4°C)

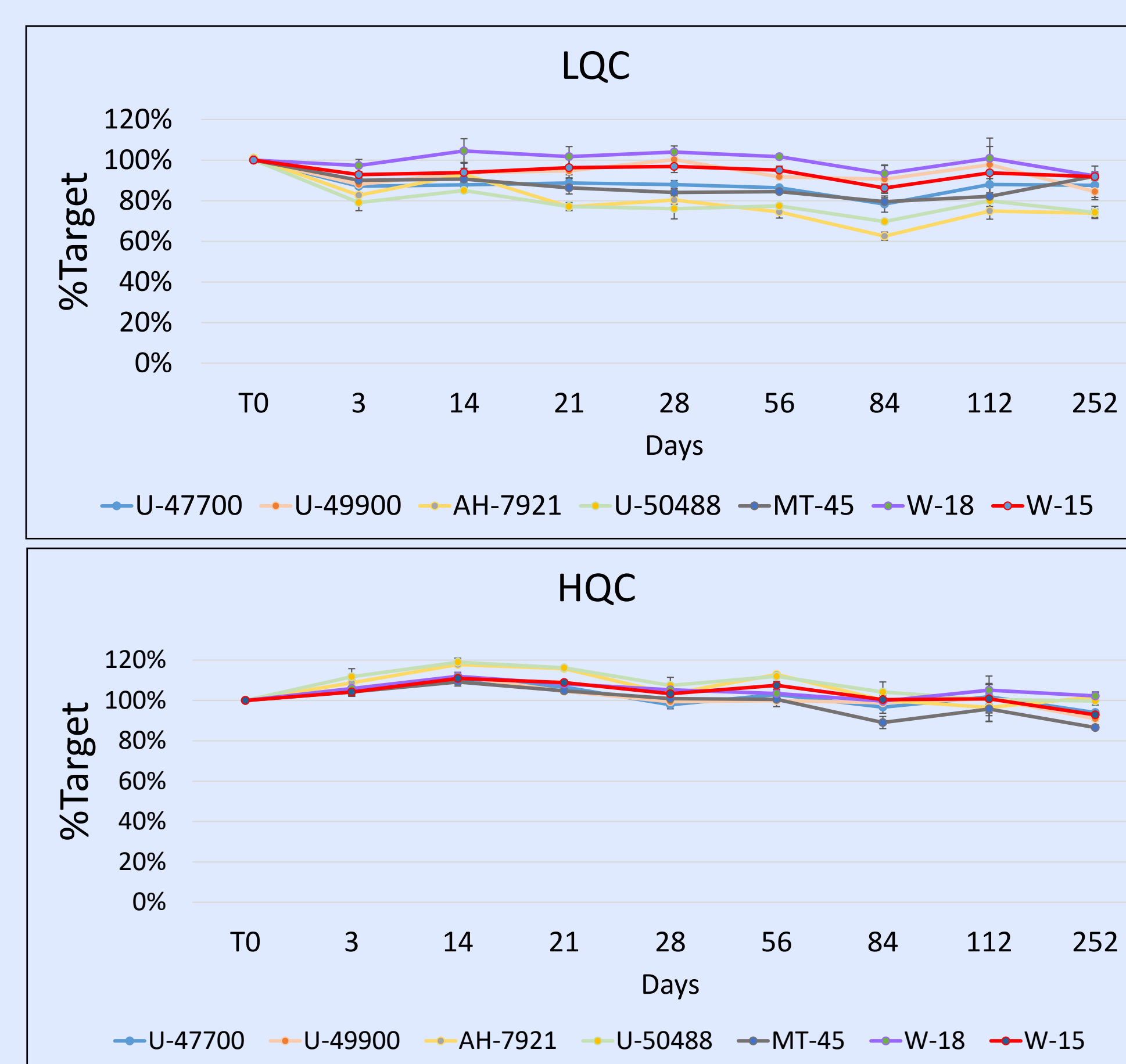


Figure 3. Refrigerated stability of U-47700, U-49900, AH-7921, U-50488, MT-45, W-18, and W-15 at a) 0.75 ng/mL (2.5 ng/mL for W-18) (LQC) and b) 80 ng/mL (HQC)

### High Temperature (35°C)

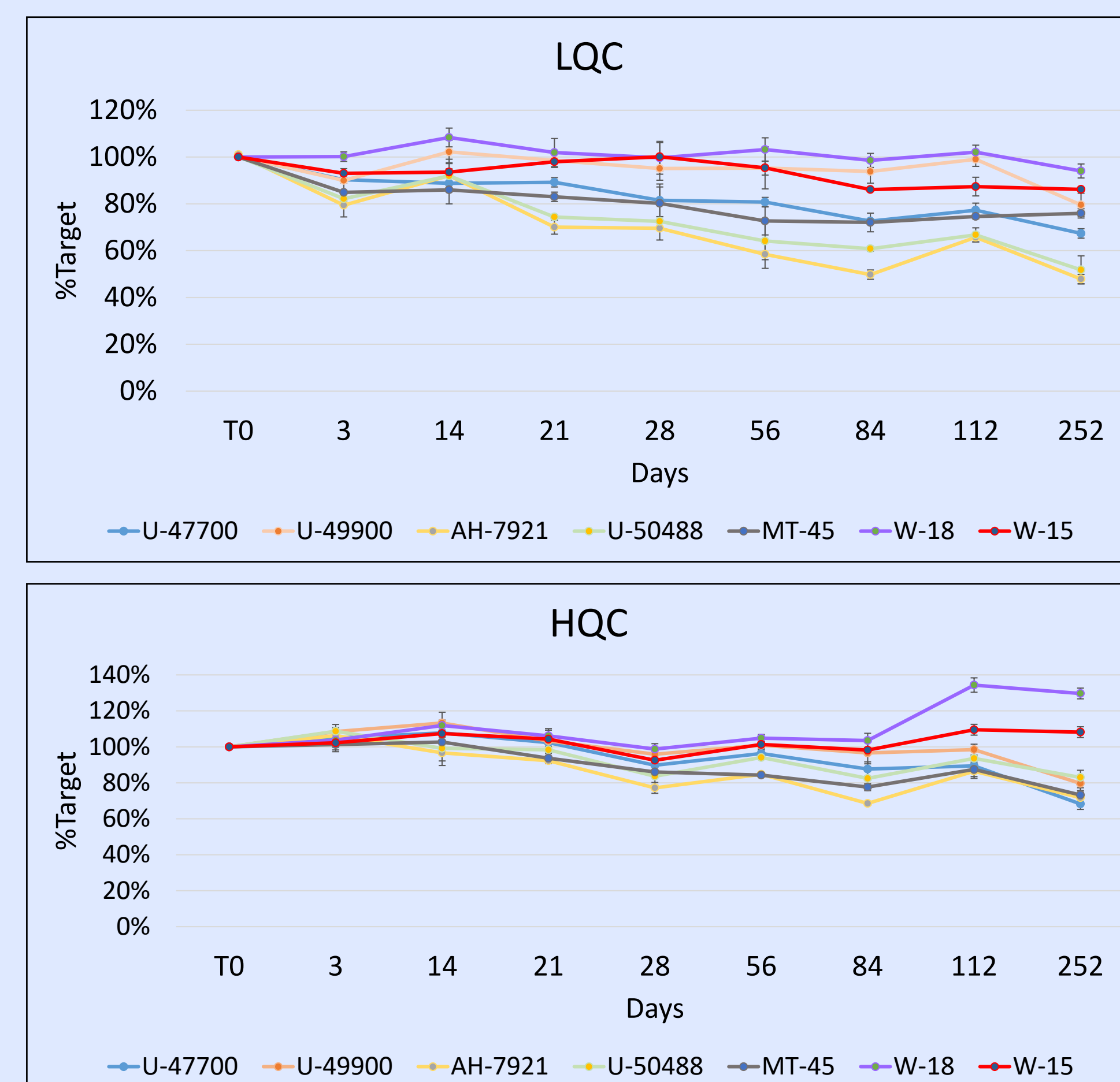


Figure 5. Elevated temperature stability of U-47700, U-49900, AH-7921, U-50488, MT-45, W-18, and W-15 at a) 0.75 ng/mL (2.5 ng/mL for W-18) (LQC) and b) 80 ng/mL (HQC)

All analytes were stable at the 4 evaluated conditions in blood for two weeks at the low concentration. There was variability observed in the low concentration for AH-7921, U-50488, and MT-45. In general, most analytes were stable at the high concentration throughout the study with the exception of the elevated temperature. Additional experiments were performed to assess increasing concentrations observed with the W-series analytes. Matrix effects in heated blood resulted in greater suppression of W-18 internal standard over time. An additional discovery during this evaluation was that there was a difference in concentrations between vacutainers that had been opened compared to those that were sampled for the first time. Multiple vacutainers were placed in each temperature setting and were used until depleted. Therefore, when switching to a new tube, there would be variability in the concentration. Although the difference was not large, this could contribute to the variability of analytes at the low concentration and may not necessarily indicate true degradation.

## CONCLUSION

- This study determined the stability of several NSO at various temperatures over a 36-week period.
- To the authors knowledge, this is the first long-term stability study involving these seven NSO and the data support the forensic significance of proper sample storage.
- The results showed minimal effect on stability at the elevated temperature during the first two weeks, indicating that these analytes would be stable in the event of improper transport/handling within this timeframe.
- Blood samples suspected to contain synthetic opioids, particularly at low concentrations, should be stored refrigerated or frozen, when possible, in order to preserve analyte stability. Samples should be analyzed within 4 weeks in order to capture true concentrations, although analyte loss did not exceed 40% was beyond this timeframe.

## REFERENCES