Problem 2

A Swedish research group, headed up by Carl Sennbro is developing a method to monitor the concentration of a new pharmaceutical to be used in the treatment of multiple sclerosis. The drug being tested is called laquinimod (also known as ABR-215062) and it is showing considerable progress in suppressing active lesion development by the disease. The clinical challenge is to determine the dose regimens for patients and an accurate knowledge of the kinetics of the drug’s metabolism is critical. In order to pursue these pharmacokinetics studies, they have developed two techniques, using tandem mass spectrometry following a liquid chromatography separation, to make these measurements. (You can see their paper in Sennbro, Olin, Edman, Hansson, Gunnarsson, and Svensson, *Rapid Communications in Mass Spectrometry, 20*(22), 3313-3318 (2006).)

Their procedure is to use an internal standard approach. The chemical structure of laquinimod is

![Chemical structure of laquinimod](image)

The internal standard is the isotopic variant formed by replacing the carbon atoms in the phenyl ring which are indicated with an asterisk with $^{13}$C. This isotope is chemically identical but readily differentiated from the analyte in the mass spectrum. The analyte shows two parent ion peaks at 357.1 and 359.1 Da (or amu); the two peaks are due to the natural abundance ratio of the chlorine atom (both $^{35}$Cl and $^{37}$Cl). The isotopic internal standard showed peaks at 363.2 and 365.2. The instrument used electrospray ionization for the ionization source and these ions were therefore monoprotonated. The spectrometer was a triple quadrupole.

The data is in the accompanying Excel or pdf files. 9 calibration standard solutions (having known concentrations for both the laquinimod and its $^{13}$C analog) were analyzed and the relative peak area is reported for both the 357.1 Da peak associated with laquinimod and the 363.2 peak for the internal standard. 4 measurements were made for each standard solution. Make a calibration curve from this data. Then two unknown samples were obtained. The internal standard was added to form a known concentration. The signal at the two m/z peaks was measured 6 times for each unknown. Determine the concentration and the 95% confidence limits for these unknowns.

If you had not used an internal standard, what would the answer (with confidence limits) have been?