High performance liquid chromatography is an analytical method that is typically used to separate components of mixtures. The separation is based on the components’ interaction with both a mobile phase and the particles in a stationary phase column. However, when it is used in conjunction with an analytical standard, it can quantify a specific analyte in a matrix. Various water samples were collected from Spring Lake in Macomb, Illinois and were extracted and analyzed for penicillin. This particular pharmaceutical has been known to be prevalent in water in trace amounts. Penicillin is one of the most common antibiotics prescribed to patients experiencing a variety of symptoms. It was one of the first known drugs to effectively treat both staph and strep infections. Less than 1% of the population experiences adverse effects from this antibiotic and are likely attributed to allergic reaction. For these reasons, quantifying penicillin in local water samples is of great interest and is the focus of the study.