



K32 The Evaluation and Preservation of Urine Specimens in Forensic Toxicology

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After attending this presentation, attendees will better understand how the pH of urine changes over time, the effect temperature has on pH change, the need for a preservative in urine samples, and the recommended minimum concentration of preservative required to stabilize urine pH. This research will directly benefit stability studies conducted on urine samples, as well as benefiting forensic and clinical laboratories analyzing for illicit substances in urine.

This presentation will impact the forensic science community by providing urine pH stability for 200 days as well as urine pH stability at room temperature from a multitude of participants to illustrate how variable the matrix is. Attendees will also understand the effects of added buffers, as well as preservatives, in varying concentrations in order to maintain the pH of a urine sample. The results of this study will allow toxicologists to better understand why some of their extractions have failed and also allows urine samples to be stored for longer periods of time to decrease the number of samples canceled for matrix stability problems. These results were evaluated for clinical and postmortem situations so a standardized method could be applied to both fields.

Urine is a commonly encountered matrix when screening for illicit substances in Driving Under the Influence of Drugs (DUID) cases within forensic laboratories and for clinical testing within hospital laboratories. Once a sample is received, after a period of storage, it generally undergoes an extraction procedure to remove any interferences from the matrix prior to instrumental analysis. Solid phase extraction techniques require pretreatment of samples to achieve an appropriate pH so the analyte of interest is in the appropriate form. This is generally achieved by use of a buffer. If the sample is not in the proper form, poor or no recovery may be a result.

During method development for a range of cathinones, it was determined that the pH of urine was changing over time and affecting these processes. It was determined that as the time a sample remained at room temperature increased, so did the urinary pH. This was hypothesized to be due to the breakdown of urea and creatinine into ammoniated compounds. This hypothesis was tested using Nessler's reagent and the Jaffee test. Nessler's reagent was used to measure the amount of urea present in a sample by scanning all wavelengths and reporting the absorbance at a specific wavelength. The Jaffe test was conducted in the same manner, but this test measured the amount of creatinine present in a sample. Appropriate calibration curves were made and urine samples were monitored over time to determine how the levels of these two compounds changed.

The addition of an appropriate preservative or buffer that can be added to urine to stabilize the pH of the matrix was investigated to help decrease the number of failed extractions and increase the stability of drugs present in urine over time. Sodium fluoride at 0.2% and 2.5% weight by volume (w/v) were added to urine samples and the pH was monitored to see if this was appropriate. Buffers of varying molarity were also evaluated. Samples were monitored in duplicate when stored in both the refrigerator and at room temperature for a period of 200 days. It was found that sodium fluoride at 0.2% w/v helped to maintain the pH with the necessary pH range required for successful extraction for a period of 90 days. Urine samples containing sodium fluoride at 2.5% w/v were found to be significantly less stable than that of the 0.2% w/v, but more stable than that of unpreserved urine. Without the addition of the preservative, urine pH is only stable for approximately two weeks. With the implementation of this research into case work, laboratories have the potential to extend the viability of the matrix and decrease the number of specimens canceled due to matrix instability. This research also provides the potential for detecting illicit substances for longer periods of time because pH is not adding to the degradation.

Urine, pH, Preservative