



K38 Development of a Method for Detecting Papain in Adulterated Urine Samples

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After attending this presentation, attendees will become familiar with a newly developed enzymatic assay for detecting papain in adulterated urine samples.

This presentation will impact the forensic community by providing a method to detect papain, a novel adulterant, in urine samples. Papain testing by this method can contribute to determining the validity of urine samples and will diminish the likelihood of individuals obtaining false negatives during drug screening due to the presence of papain in urine.

Papain is a novel urine adulterant being used to interfere with the common drug screening methods used in urine drug testing. In a study by Burrows et al., papain was found to interfere with the analysis of some drugs and was not detected in urine using current guidelines of specimen validity testing.¹ Thus, a method is needed to detect papain in urine and contribute to rendering the urine sample invalid. The current research developed an enzymatic assay for detecting papain in urine samples.

Papain is a cysteine protease that has a broad specificity, cleaving peptide bonds involving basic amino acids, leucine, and glycine. It hydrolyzes both esters and amides. A synthetic substrate, N α -Benzoyl- DL-arginine-4-nitroanilide (BANI), was used in assay development. Papain acts on BANI to release p-nitroaniline which absorbs at 410 nm. The rate of formation of this product is easily monitored by following the change in absorbance at 410 nm. Assay conditions were established. Experiments examining the rate of product formation with varying papain concentrations and found that a linear relationship existed between the rate of product formation and concentration of papain. Papain activity as low as 0.003 units could be detected by this method. Unknown blind samples of papain were analyzed and were accurately determined as being either positive or negative for papain using this method. **Papain itself is available from a variety of vendors and there are currently no restrictions on possession or use of papain, which makes it an easily accessible urine adulterant. However, there are also common consumer products that contain papain such as Adolph's[®] Meat Tenderizer and Beverly International[®] Multiple Enzyme Complex which is marketed as a digestive supplement.** The assay could also detect papain in urine when these consumer products were the source of papain. The effect of storage conditions on papain activity in urine was also examined. Storage for one hour at room temperature had no significant effect on papain activity. Storage at room temperature for 2 h to 24 h led to a decrease in activity ranging from a 22% decrease at 2 h to a 48% decrease at 24 h. Storage at 4^o C for 2h to 24 h led to a decrease in activity ranging from 22% at 2 h to 52% at 24 h. The results of testing for potential interference by common drugs of abuse in this papain assay will also be presented.

Reference:

- ¹ Burrows, DL, Nicolaides, A, Rice, PJ, Dufforc, M, Johnson, DA. Papain: A Novel Urine Adulterant. Journal of Analytical Toxicology 2005;29:275-295.

Papain, Adulterant, Urine Drug Testing