Enzymic Digestion of Biological Specimens for Drug Analysis

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ABSTRACT: In the first part of this paper, procedures using β-glucuronidase for the analysis of drug conjugates in urine are reviewed and compared to the more classical techniques of acid hydrolysis. Morphine-3-β-D-glucuronide is one of the most commonly encountered drug conjugates that may be either acid hydrolyzed or enzyme hydrolyzed to yield free morphine. Because of its rapidity of method and high yield of drug, acid hydrolysis of morphine glucuronide is still the most popularly employed technique for the release of free morphine. However, enzyme hydrolysis has been shown to enjoy virtually the same rapid hydrolysis and high yield of free drug, and it does not hydrolyze acid-labile drugs that might be present. In the second part of this paper, a discussion of the benefits of enzyme hydrolysis of tissues, hair, etc., for the release of drugs is presented. Such benefits include milder conditions, improved yields of drug, cleaner extracts, and the prevention of emulsions, especially when compared to acid hydrolysis. Several proteolytic enzymes have been employed for tissue digestion, including pepsin, subtilisin Carlsberg, papain, trypsin, neutrase, and others. While no one enzyme system has surfaced as being clearly the most effective in releasing drugs from tissues, several have been found to be quite effective, e.g., the proteolytic enzyme subtilisin Carlsberg. The proteolytic enzyme papain has also been recommended because of its long shelf life, low cost, and excellent recovery of drugs from tissues.

KEY WORDS: Acid hydrolysis, drug analysis, drug conjugate, enzymic digestion, enzymic hydrolysis, β-glucuronidase, subtilisin.