ASSESSMENT OF RECOMBINANT BETA-GLUCURONIDASE *VS* NATIVE BETA-GLUCURONIDASE AND ALKALINE CONDITIONS FOR THE HYDROLYSIS OF THC-GLUCURONIDE

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SCAN ME

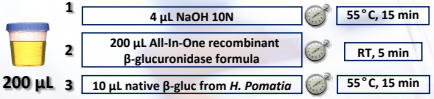
INTRODUCTION

The use of enzymes plays an important role in the hydrolysis of conjugated molecules during the preparation of biological samples, especially urine. Several clinical and forensic applications, including Therapeutic Drug Monitoring (TDM) and toxicological analyses, can take advantage of an effective, easy, time-saving procedure. In this perspective, we aimed to assess the yield of two different beta-glucuronidase enzymes, one recombinant and one native, in comparison with the alkaline hydrolysis routinely used in our laboratory for the analysis of cannabinoids in urine samples.

METHODS

A total of 82 anonymous urine samples were tested with an ISO 17025 accredited UHPLC-MS/MS method for the detection of THC-carboxylated metabolite.

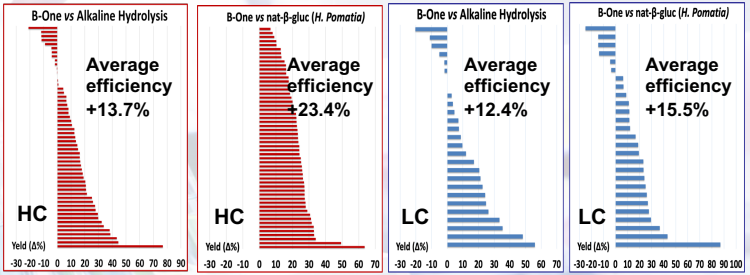
During the SAMPLE PREPARATION, three different pre-analytical hydrolysis steps were performed:



In a second experiment, further 35 anonymous urine samples were tested with different volumes (respectively, 20, 50 and 100 µL) of recombinant β-gluc, to optimize the working conditions (incubation at RT for 5 min).

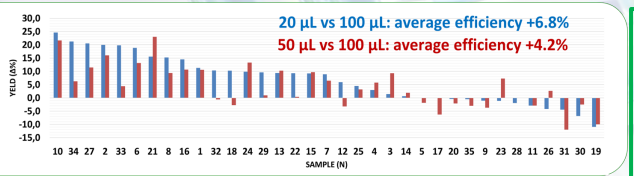
RESULTS

According to the concentrations obtained with the routine procedure, the 82 samples were divided into "high concentration" (HC, above 55 ng/mL; N = 55) and "low concentration" (LC, below 55 ng/mL; N = 27) samples.



$$\text{Yield } (\Delta\%) = ([\text{THC-COOH}]_{\text{B-One}} / [\text{THC-COOH}]_{\text{Other}}) \times 100$$

The second experiment showed that the decreasing of the B-One enzyme amount did not affect the hydrolysis efficiency proving that a minimum amount of 20 µL is enough to obtain satisfactory results.



The recombinant beta-glucuronidase provided the most efficient hydrolytic activity to cleave the glucuronide of THC-carboxylated metabolite. Further experiments are needed to verify that similar results are obtained for other glucuronate metabolites, including other cannabinoids. By achieving a high yield in only 5 minutes without a heating step, the laboratories, especially in clinical, forensic, TDM, and WDT, will be enabled to increase the number of processed urine samples, with no detriment of the analytical performances.

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