



Analytical Considerations for the Successful Evaluation of Hyoscyamine Biotransformation into 6 β -Hydroxyhyoscyamine and Scopolamine

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SUMMARY. Hyoscyamine, 6 β -hydroxyhyoscyamine and scopolamine are anticholinergic agents that belong to the tropane alkaloids, a pharmacological important group of secondary metabolites. Hyoscyamine and scopolamine were historically used in medicine. Additionally, potential medical applications for 6 β -hydroxyhyoscyamine were described in the last years. Previous works carried out in our lab allowed us the construction of a *Saccharomyces cerevisiae* strain harboring the Hyoscyamine-6 β -hydroxylase (H6H) enzyme which is responsible of the conversion of hyoscyamine into 6 β -hydroxyhyoscyamine and scopolamine. Several factors influenced and complicated the optimization of the hyoscyamine bioconversion process. The aim of this work was to evaluate the analytical factors that critically affect the performance of the alkaloid extraction and the detection and quantification method of the alkaloids implied in the biocatalytical process. The mechanical breakdown of yeast cells by continuous agitation at 4 °C in 2 mL tubes was the method of choice for an efficient recovery of the functional H6H enzyme. In addition, the different pH assayed for the alkaloid extraction caused significant variations in the recovery of the alkaloids, specifically impacting on scopolamine recovery which decreased a 35 % after the increase of the pH of the extraction. The development of robust and sensitive analytical methods was requisite for the correct monitoring and quantification of the alkaloids produced in order to evaluate the technological and economic feasibility of this process.

KEY WORDS: 6 β -hydroxyhyoscyamine, Biotransformation, Hyoscyamine, Hyoscyamine-6 β -hydroxylase, Scopolamine.

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