Short Communication

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Analytical method development for directed enzyme evolution research

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Abstract

Analytical methods were developed for a directed enzyme evolution research programme, which pursued high performance enzymes to produce high quality L-ribose using large scale biocatalytic reaction. A high throughput HPLC method with evaporative light-scattering detection was developed to test ribose and ribitol in the enzymatic reaction, a β-cyclobond 2000 analytical column separated ribose and ribitol in 2.3 min, a C_{18} guard column was used as an on-line filter to clean up the enzyme sample matrix and a short gradient was applied to wash the column, the enzymatic reaction solution can be directly injected after quenching. Total run time of each sample was approx. 4 min which provided capability of screening 4 × 96-well plates/day/instrument. Meanwhile, a capillary electrophoresis method was developed for the separation of ribose enantiomers, while 7-aminonaphthalene-1,3-disulfonic acid was used as derivatisation reagent and 25 mM tetraborate with 5 mM β-cyclodextrin was used as electrolyte. 0.35%of D-ribose in L-ribose can be detected which can be translated into 99.3% ee of L-ribose. Derivatisation reagent and sample matrix did not interfere with the measurement.

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Biography

Wan Jee Lee has completed his PhD from University of Wisconsin-Madison, USA. He now serves as an Associate Professor at the Department of Food Science in Yuanpei University of Medical Technology, Taiwan. His research interests lie in the domain of food engineering and processing technology, and he has published many biofuels-related papers in reputed journals.

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