Application of a novel HPLC-based system for automated biotransformation and analysis

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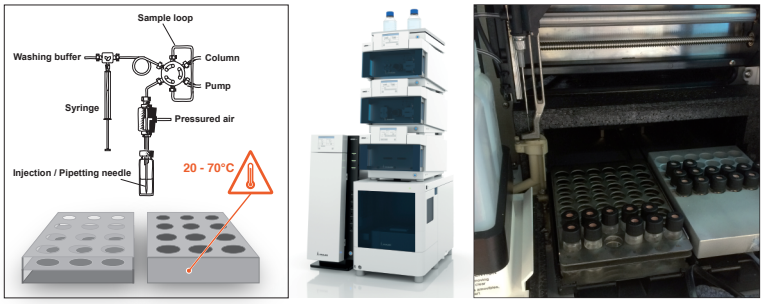
Background

In biotechnological research, small-scale approaches are reasonable due to economical benefits especially when tests involve costly substances (substrates, enzymes). In general, two steps are necessary to evaluate the performance of a biotransformation: 1) The realization and monitoring of the conversion itself and 2) the analysis of the conversion products. Additionally, a derivatization step might be necessary for analysis. In this study both steps were combined and automated by a novel HPLC-based system.



Biotransformation, derivatization and analysis

Reactions were carried out in the autosampler of the HPLC system (PLATINblue, Knauer). The enzyme glutaminase (EC 3.5.1.2) was chosen as model for the evaluation of the described system. Glutaminase catalyzes the hydrolytic deamination of free L-glutamine (L-Gln) to L-glutamic acid (L-Glu). After the biotransformation the substrate L-Gln and the product L-Glu were automatically derivatized with *ortho*-phthalaldehyde (OPA) [1] and separated by a RP-C18 column. The standard procedure for the glutamiase assay is shown in figure 1.

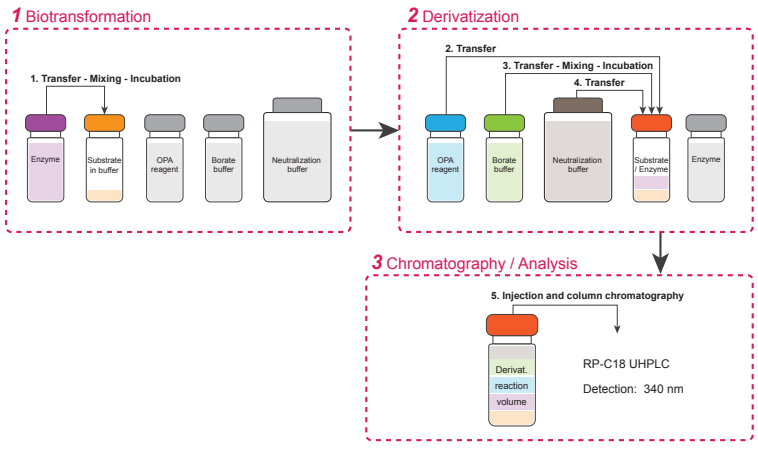


Figure 1: Pipetting scheme for the biotransformation and analysis of the glutaminase assay in the autosampler vials.

- » Transfer of enzyme to the substrate/buffer solution
- » Mixing and incubation (30°C)
- » Transfer of the sample to the derivatization vial
- » Derivatization (20°C) with OPA reagent and borate buffer
- » Neutralization of the mixture and column chromatography

Literature:
[1] Church, F.; Swaisgood, H. E.; Porter, D. H.; Catignani, G. L.; **Spectrophotometric Assay Using o-Phthaldialdehyde for Determination of Proteolysis in Milk and Isolated Milk Proteins;** *Journal of Dairy Science;* 1993; 66:1219-1227

Proof of principle

The obtained Michaelis–Menten enzyme kinetic of the glutaminase, which was assessed with the described system, is shown in figure 2. The K_M value was 520 µM and a v_{max} of 1.57 nkat mg^{-1} was determined after linearization according to Hanes. The complete enzyme kinetics were measured in duplicates without the expenditure of manpower in less than 4 h.

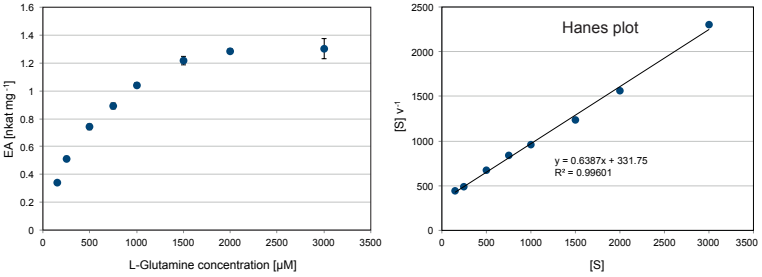


Figure 2: Glutaminase kinetics determined with the automated HPLC system.

Key features of the automated system

- » Up to 5 different solutions can be used for the biotransformation and analytic steps
- » The present system can also be used for peptidase assays. In previous studies 18 amino acids were separated by RP-HPLC after precolumn derivatization with OPA (figure 3)
- » Independent temperature zones are manageable for biotransformation and derivatization reactions

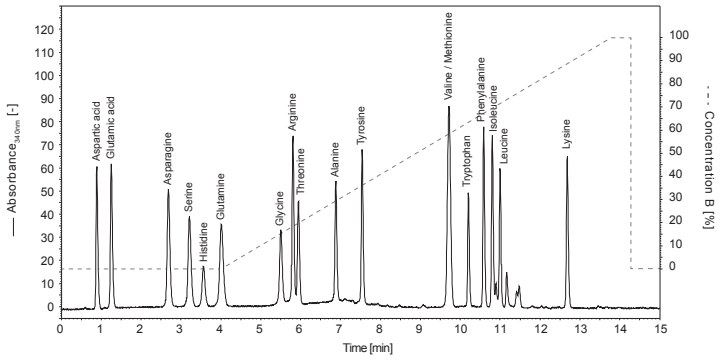


Figure 3: Analysis of amino acids (OPA derivatives) by RP-HPLC.

Outlook

- » The system possesses high versatility and will be used for biochemical characterizations of enzymes
- » Biotransformation and derivatization parameters (temperature, pH) can be set independently
- » Automated three-step biotransformation, derivatization and subsequent analysis of the products were achieved in less than 15 min per sample
- » Efficient use of manpower and time

Acknowledgements: The authors would like to thank Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer GmbH, Berlin (Germany) and Dipl.-Ing. (FH) Wolfgang Claaßen for their technical support.