On-line detection of enzyme inhibitors using high-performance-liquid-chromatography hyphenated to a biochemical detector

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Enzymatic conversions play an important role in the regulation of all processes of life. For example proteases like thrombin or angiotensin converting enzymes are indispensable in the regulation of blood coagulation and blood pressure. Hence, inhibitors could be applied as drugs e.g. for the treatment of hypertension and thrombosis. On the other hand, the inhibition of enzymes could lead to adverse effects. For example the inhibition of phase-II metabolism enzymes like glutathione-S-transferase (GST) could lower the physiological detoxification dramatically. Hence, it is from pharmaceutical as well as toxicological relevance to analyze e.g. food ingredients, extract from plants, bacterial suspensions for the presence of enzyme inhibitors. To identify the active compounds in these complex mixtures a separation step prior the detection of the inhibitory activity is needed.
Therefore, we are developing methods, which combine HPLC separation with the biochemical detection (BCD) of the enzyme inhibition (Fig.1).

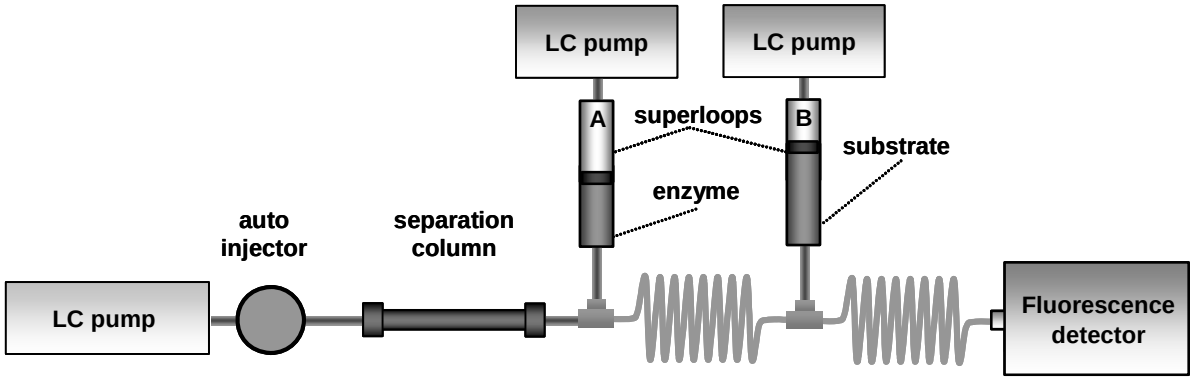

Fig. 1: Schematic view of the biochemical detection system [1]

Here, the mixture containing potential inhibitors can be directly injected into the system. In the first step components are separated by reversed phase HPLC. Subsequently, the regarded enzyme is added to the eluat. After a reactor coil, allowing the reaction of the inhibitors with the enzyme, the fluorescence-labelled substrate is added and converted by the enzyme. In case of eluting inhibitors, the amount of fluorescent product decreases, which can be observed as negative peaks.
Through a selective choice of substrates, this method can be applied on various enzymatic conversions e.g. for proteases with coumarin-labelled peptides [1] or for GST-inhibition with glutathione and monochlorobimane as substrates [2].

1. Schebb, N. H. et al. Anal Chem (2008, accepted for publication).
2. Kool, J. et al. J Biomol Screen 12, 396-405 (2007).