Acetylcholinesterase enzyme silica capillary tube reactor for online extracts screening

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The development of screening methods to identify new, biologically active compounds in complex mixtures is a challenging task.[1] Immobilized capillary enzyme reactors (ICERs) for on-line ligands screening have been adopted as a technique of high throughput screening (HTS) [1,2]. Here, we selected the enzyme acetylcholinesterase (AChE) that represents a widely studied target enzyme especially in the realm of drug discovery programs concerning Alzheimer's disease (AD).[3] To prepare AChE-ICERs, AChE from electric fish (*Electrophorus electricus*) or AChE from human erythrocytes, was covalently immobilized on fused silica capillary using the homobifuncional agent glutaraldehyde as spacer, which resulted in two bioreactors: *ee* AChE -ICER and *hu*AChEICER. The ICERs were coupled to a high performance liquid chromatography system with UV-vis detection.[2] The analytical method was validated for both IMERs, which were then used in a study for determination of the inhibitory potency (IC_{50}) of standard AChE inhibitors. Screening 40 plant extracts identified 15 promising active extracts of with more than 60% inhibition. The sample that afforded 90% inhibition in *ee* AChE-ICER and 58% in *hu*AChE-ICER.was selected for the purification work. Samples were also evaluated by anticholinesterase solution assay and false positive assay for comparison purposes which evidenced high similarity. Results revealed that this method is suited to the identification of the inhibition activity of more complex mixtures without the need for additional pre-fractionation. This is a fast and useful approach for the accurate and reproducible automated screening of synthetic or natural ligands. Moreover, these ICERs are a great alternative for the preservation of the enzymatic activity (retention of 78% initial enzymatic activity for 15 months for - *ee* AChE-ICER and 60 days for *hu* AChE- ICER).

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Literature

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