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Identification of bioactive compound from natural products: on-line HPLC hyphenation with enzyme reactors and MS detection

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High-throughput and efficient analytical approaches for bioassay-guided fractionation and chemical profile of crude extracts is still challenging. Nowadays researches are focusing on advanced hyphenated techniques that enable the direct identification of compounds with the desired biological effect like on-line high-performance liquid chromatography (HPLC) with *in vitro* biochemical assay. The use of immobilized enzyme reactors (IMER) as an on-line post-chromatographic biochemical assay is a promising alternative. Immobilization procedure can increase enzymatic stability against heat, organic solvent and pH without too much loss of catalytic activity allowing the direct coupling to a liquid chromatographic separation system using a sampling valve as interface. Our group proposed for the first time an on-line comprehensive *in vitro* bioassay using an immobilized capillary acetylcholinesterase reactor (ICER-AChE) coupled to mass spectrometry detection. This, proved to be a powerful method for the fast screening of inhibitors in natural products crude extracts. Two well established acetylcholinesterase inhibitors, galantamine and donepezil, were used as proof of concept. The innovative two-dimensional developed system allowed recognition of both compounds as acetylcholinesterase inhibitors by direct monitoring acetylcholinesterase hydrolysis product choline's (Ch) precursor ion m/z 104.0 ($[M+H]^+$) and its fragment m/z 60.0 ($[M+H-C_2H_5OH]^+$).¹ The combination of the analytical separation with on-line AChE enzymatic assay coupled to mass spectrometry increases the likelihood of identifying individual inhibitors compared to conventional assays reducing considerably the time required to assign a biology activity to a chemical structure.

Key-words: immobilized enzyme reactor, AChE, screening.

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Reference:

¹Vanzolini, K.L., Vieira, L.C.C., Correia, A.G., Cardoso, C.L., Cass, Q.B., 2013. Acetylcholinesterase immobilized capillary reactors-tandem mass spectrometry: An on-flow tool for ligand screening. *J. Med. Chem.* 56, 2038-2044.