

Comprehensive two-dimensional LC-MS based acetylcholinesterase immobilized capillary reactor for the screening of inhibitors in natural products

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Enzymes packed into columns and used in a flow system as immobilized enzyme reactors (IMER) are well suited to high throughput screening (HTS) for the fast hit inhibitors selection in the early discovery process suitable to analyze pure compounds or simple mixtures [1, 2]. The immobilization procedure can increase the enzymatic stabilization against heat, organic solvent and pH without too much loss of catalytic activity. On-line coupling of IMERs to separation methods such as high performance liquid chromatography (HPLC) can be better suitable in natural products-based drug discovery program applied to complex matrices such as crude extracts where the chromatographic separation is followed by IMERs bioactivity detection [3]. Here the on-line combination of comprehensive two-dimensional liquid chromatography (LCxLC) with the immobilized capillary acetylcholinesterase reactor was investigated as a powerful method for the screening of inhibitors in natural products. Our group proposed the potential use of one-dimensional RP-LC separation and an off-line comprehensive HILICxRP-LC (Fig. 1) separation of two different methanol extracts from bulbs and leaves an Amaryllidaceae specie endemic in Brazil (*Hippeastrum calyptratum*) coupled on-line immobilized capillary acetylcholinesterase reactor inhibition assay-tandem mass spectrometry. The activity and kinetics parameters were evaluated by monitoring the choline's precursor ion $(M + H)^+$ m/z 104.0 and its on fragment $(C_2H_3OH)-(M + H)^+$ m/z 60.0. The assay method was validated using the reference AChE inhibitors tacrine and galanthamine. The combination of LCxLC separation with on-line AChE enzymatic assay-tandem mass spectrometry increases the likelihood of identifying individual inhibitors compared to conventional assays.

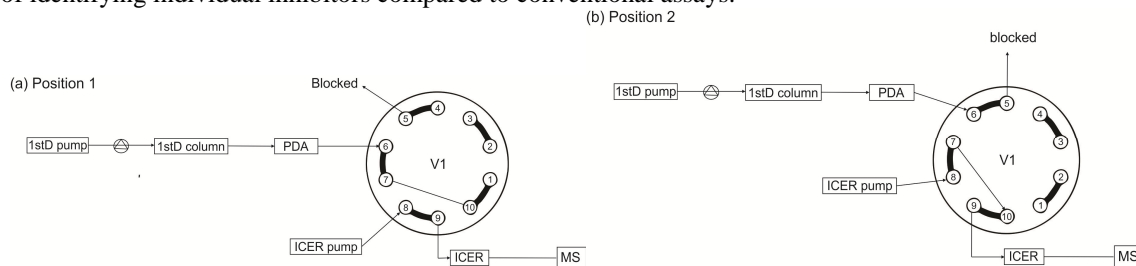


Figure 1. A schematic illustration of the instrumental configuration used for one-dimensional LC-ICER-MS and off-line HILICxRP-LC-ICER-MS analysis.

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