

Affinity selection mass spectrometry (AS-MS) assay using Monoamine oxidase A and B for ligand screening of *Aloysia polystachya* extract

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Highlights

The enzymes monoamine oxidase (MAO) A and B were covalently immobilized on magnetic beads (MBs). In the affinity-based assay compounds that bind to the immobilized targets are retained and can be eluted for further structural analysis.

Abstract

The enzymes monoamine oxidase A (MAO-A) and B (MAO-B) play key roles in regulating neurotransmitters such as dopamine and serotonin¹. Dysregulated activity of these enzymes contributes to neurological disorders, including Parkinson's disease and depression²⁻³, making them attractive targets for identifying molecules that can modulate MAO activity. The screening assay model, using affinity selection mass spectrometry (AS-MS), involves an immobilization procedure followed by an incubation step (S0), washing to separate binders from non-binders (S1-2), binder desorption (S3), and subsequent structural characterization.⁴ Analytical approaches based on LC-MS are commonly employed to obtain the chemical profile of ligands with affinity for the immobilized target protein. Here, we present an affinity-based assay, also known as ligand fishing, using MAO-A and MAO-B individually immobilized on magnetic particles (MBs) via the amine-glutaraldehyde reaction, producing MAO-A-MB and MAO-B-MB. This method was applied to identify binding molecules from the acetate extracts of *Aloysia polystachya*, which has shown inhibitory activity against both MAO-A and MAO-B and possesses reported pharmacological properties such as antidepressant and anxiolytic effects⁵. Ligands with an affinity ratio (AR)* > 1.0, detected during the desorption step, were selected as strong binders. The samples containing these ligands were analyzed by untargeted mass spectrometry, and the LC-MS/MS data were searched against the spectral library from the Global Natural Products Molecular Networking (GNPS). Through comparison with public databases, 38 metabolites were annotated in the *A. polystachya* extract. These metabolites belong to different chemical classes, including glycosylated benzoic acid, flavonoids, terpenoids, and fatty acids. Based on the affinity ratio (AR) calculations, the following ions were considered potential ligands for MAO-A: *m/z* 345 (AR = 1.59 ± 0.07), *m/z* 315 (AR = 1.36 ± 0.05), and *m/z* 609 (AR = 1.22 ± 0.05). These ions were annotated as Eupatorin, Cirsimaritin, and 10-Hydroxypheophorbide A, respectively. Additionally, 10-Hydroxypheophorbide A also exhibited an affinity ratio for MAO-B, with AR = 1.51 ± 0.19.

*AR = A_i/A_0 (A_i is the area of the ligand peak obtained in fraction S3 with IMER-KLK-MP. is the area of the ligand peak obtained in fraction S3 with the control).

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