A novel screening method for ALOX5 inhibitors based on TLC plate and enzymatic assay

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Résumé

In 2021, according to the "Assurance Maladie", almost 4 million French people were suffering from chronic inflammatory diseases, representing a major public health challenge(1). Arachidonate 5-lipoxygenase (ALOX5) plays a central role in the pathogenesis of several of these diseases, including asthma, rheumatoid arthritis and Crohn's disease. ALOX5 catalyses the production of leukotrienes, the pro-inflammatory lipid mediators that trigger the inflammatory response responsible for various pathologies. This involvement makes ALOX5 a prime therapeutic target for the development of new anti-inflammatory drugs. At present, Zileuton is the only drug approved to specifically inhibit this enzyme, and only in the United States(2). Available ALOX5 inhibitor screening assays rely mainly on expensive commercial enzyme kits using chromogenic or fluorogenic reactions, which are not suitable for complex plant extract screening.
In contrast, enzymatic assays coupling thin-layer chromatography (TLC) separation with *in situ* enzymatic reactions have been developed for the screening of enzyme inhibitors such as cyclooxygenase 2(3), tyrosinase and acetylcholinesterase, offering direct and rapid visualization of enzyme inhibition. TLC assays coupled with an *in situ* enzyme assay are also suitable for screening complex plant extracts(4).

Here, we present the development of a simple, rapid and cost-effective enzymatic assay on TLC plates for detecting ALOX5 inhibitors. The test is based on the oxidation of a colourless reagent during the enzyme conversion of arachidonic acid to leukotriene, generating an electrophilic intermediate that will complex with a second reagent to form a grey-coloured compound. The grey colouration indicates the enzyme activity, while zones of inhibition appear as white spots on the grey background. The method was developed and validated using three positive inhibitors: zileuton, nordihydroguaiaretic acid, and piperine, as well as two negative controls: caffeine and salicylic acid(5). This TLC-based enzymatic assay provides an efficient and accessible alternative for the preliminary screening of ALOX5 inhibitors, facilitating the discovery of new anti-inflammatory agents, particularly from complex plant extracts.

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Mots-Clés: Arachidonate 5, lipoxygenase, thin layer chromatography, in situ enzyme assays, inhibitor screening, complex plant extract.