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Dipeptidyl peptidase IV (DPP IV) inhibitors from plant extracts by TLC bioautography

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TLC bioautography is an assay that combines chromatographic separation and *in situ* determination of biological activity. It has advantages over other screening methods for its ability to screen mixtures (a wide solubility range of compounds) simultaneously. In addition, it can reduce the cost and time of screening. TLC autobiography assays have been established for screening compounds for antimicrobials, antioxidants (free-radical scavengers) and enzyme inhibitors. In this presentation, the application of TLC bioautographic techniques on enzymatic reaction will be reviewed. An example of the development of a novel TLC-bioautographic method on detection of dipeptidyl peptidase IV (DPP IV) inhibitors from plant extracts will be described. The focus of the example will be on how reaction products via derivatization can facilitate such screening technology. In this case, the enzyme (DPP IV) hydrolyses substrate (Gly-Pro-p-nitroanilide) into p-nitroaniline (pNA), which diazotizes with sodium nitrite, and then reacts with N-(1-naphthyl) ethylenediamine dihydrochloride in turn to form a rose-red azo dye which provides a rose-red background on the TLC plates. The DPP IV inhibitors showed white spots on the background as they blocked enzymolysis of the substrate to produce pNA. The method was validated with respect to selectivity, sensitivity, linearity, precision, recovery, and stability after optimizing key parameters including plate type, time and temperature of incubation, concentration of substrate, enzyme and derivatization reagents, and absorption wavelength. One natural compound harmine was isolated and identified to be a potential DPP IV inhibitor in nine medicinal herbs by this method.

Biography

Lihua Gu is currently a PhD student at Shanghai University of Traditional Chinese Medicine. Her research interest includes the research and application of HPTLC, TLC-bioautographic methods and quality control standard in TCM. She has published more than 10 academic papers.

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