Thin-Layer Chromatography Bioautography

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Thin-layer chromatography (TLC) bioautography is a methodological technique that integrates the separation and analysis technology of TLC with biological activity detection technology, which is used to isolate, locate and evaluate the active constituents of natural creatures based on the guidance of activity.

Keywords: thin-layer chromatography ; bioautography ; segregation analysis ; activity detection ; practical application

1. Introduction

There are abundant resources of animals, plants and microorganisms in nature, from which the screening and extraction of bioactive substances has always been the focus of research at home and abroad. The chemical constituents in biology are manifold and structure utterly different, but which with biological activity are less proportion. Therefore, detecting and screening of biological active components rapidly is vital to the study of natural active substances. However, the traditional method of separation of active ingredients from natural products also need to pass to isolate compounds in natural products and screening of the compound active two step, the activity of general appraisal should be carried out in the entire history of the animals or organ, so often appear isolated compounds for the active substance or quantity is not enough to a large number of active screening[1].Consequently, to establish a rapid and accurate screening method for active substances plays an important role in the separation of active substances, the discovery of new compounds, the control of quality standards and the expansion of the application range of components.

TLC bioautography is used to isolate, locate, and evaluate the active constituents of natural creatures based on the guidance of activity refers to a methodological technique, and has the advantages of operation simply, cost lowly and high levels of sensitivity and specificity. This technique combines TLC separation with biological activity determination, TLC is a modern chromatography technology in which mixtures are separated by adsorption materials coated on the surface of the supporting plate as the stationary phase and eluent as the mobile phase. By means of utilizing the different adsorption capacities of each component on the stationary phase, continuous adsorption and desorption can be generated during the mobile phase elution process to achieve the separation of different component. Bioautography is a method that uses chemical components to react with substrates and chromogenic agents to form disparate color contrasts, so as to observe contrast spots in a chromogenic background to track active components [2]. TLC combined with bioautography can reduce the blindness of compound separation and reduce the use of active animals compared with traditional methodology. On the one hand, it can reflect the difference of chemical composition between samples. On the other hand, this assay format allows preassigning of the biological activity, observed in a mixture, to one or a few of its components through the spots that they produce [3]. It determines the presence, absence or strength of the biological activity of the detected substance by showing spots with different colors from the inactive area under certain conditions, which is especially suitable for the screening and discovery of active ingredients in complex mixture systems.

2. Classification

Three basic types of TLC bioautography can be distinguished: agar diffusion, direct bioautography and agar overlay bioautography, and, in addition, HPTLC bioautography and 2D-TLC bioautography, which have emerged in recent years.

Agar Diffusion, also known as the agar contact method, agar diffusion is also the least-employed one of these techniques. In this method, medium inoculated with pathogenic microorganisms is used as the carrier, and a thin-layer plate adsorbed with the compounds is face down and in contact with the surface of the medium. The compound is placed in contact with the surface of the medium for a certain time to diffuse it, and, then, after removal of the thin-layer plate, the medium is cultured overnight for bioautography.

Direct bioautography (DB) is the most commonly used and easiest method among the three methods. This method, using a thin-layer plate as the carrier, sprays the pathogenic microorganism suspension with a certain concentration of specific nutrient solution directly onto the unfolded thin-layer plate, or directly places the plate into the suspension. It can observe the experimental result directly or under the appropriate color reagent after cultivation in a humid and dark environment for a period of time.

Agar Overlay Bioautography, also known as immersion bioautography, agar overlay bioautography is a hybrid of both previous methods [4]. In order to allow a good diffusion of the tested compounds into the agar medium, the plates can be placed in a low temperature for a few hours before incubation. The melt agar medium inoculated with microorganisms is uniformly coated on the thin plate. After the agar solidifies, the thin plate is cultured overnight and dyed with a color developing agent, and then the experimental results can be observed [5]. The advantage of this method is to reduce the influence of the experimental steps on the results.

3. Detection Technique

TLC bioautography can only select materials that contain active ingredients, and it is therefore crucial that follow-up tests are performed for the determination of specific components. Commonly used techniques to determine the structure of the compound technology involves ex situ and in situ techniques; the former mainly includes nuclear magnetic resonance (NMR), electron ionization mass spectrometry (EI-MS) and electrospray ionization mass spectrometry (ESI-MS), which are common detection techniques and suitable for the detection of most compounds. Traditional MS mostly uses closed ionization technology, which requires complex pretreatment operation before sample analysis, which limits the wide application of this technology. In situ ionization MS is a kind of direct ionization and mass analysis technology for samples under normal temperature and pressure conditions without sample pretreatment [6]. The advent of this technology is a major change in the field of mass spectrometry analysis, which can realize in situ, rapid, nondestructive and direct mass spectrometry analysis of trace components on the surface of objects, greatly expanding the application range of mass spectrometry analysis. In situ ionization mass spectrometry can be used to identify the structure of chemical components on the thin-layer surface; solve the problem of compound analysis after the active components are screened out by TLC bioautography; and realize in situ, real-time and direct mass spectrometry analyses of target components in spots with inhibitory effects under the background of active color on the thin layer. In particular, the direct analysis in real-time mass spectrometry (DART-MS) and desorption electrospray ionization mass spectrometry (DESI-MS) developed in recent years have attracted extensive attention for their rapid in situ analysis of samples free of preparation.

4. Biological Applications

At present, the commonly used microbiological activity determination methods include the microbial inhibition method, enzymatic method, immunoassay method, radio immune assay (RIA) method, etc. These methods have their own advantages and disadvantages. For example, although the microbial inhibition method is simple in operation and low in cost, its specificity and sensitivity are not high, and it is easy to produce false positive results. However, highly sensitive, enzymatic and radio immune assays are expensive [7]. Broth microdilution method is a commonly used method for screening compounds with antimicrobial activity, it needs to co-culture the compounds with the culture medium and microorganism, which take a lot of time and narrowed the scope of application, which is used to detect bacterial or fungal resistance and drug sensitivity[8][9]. Compared with the above methods, TLC bioautography is an excellent method for the determination of biological activity. It is possible with a minimum amount of laboratory equipment and apparatus, operation simpler, experimental cost lower, sensitivity and specificity higher, at the same time, the bioactivity of metabolites or converts of compounds can be determined by TLC bioautography. However, TLC bioautography also has certain limitations. One such obstacle is the insensitivity of cytotoxic compounds, such as camptothecin, quassinoid and lignans, most of which are difficult to show inhibitory spots on the thin-layer plate. In addition, as the microbial culture medium used by TLC bioautography is generally used as a solvent with water, the polarity is relatively large, so when the measured compound is too polar, it is easy to spread too fast, then lead to difficult to form inhibition spots, questions regarding the validity of too small polarity results in compounds is a barrier to the application in this technology, because of they are not easy to enter the medium and also cannot form inhibition spots. This defect can be improved by 2D-TLC bioautography established later.

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