

Methodology of determination of ingredients in Natural Products

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Abstract

Determination of ingredients in natural products can help evaluate the efficacy and toxicity of natural products by dose-activity dependent relation of their ingredients. It is important for quality control of natural products. There are two major approaches for determination, horizontal approach and vertical approach. Horizontal approach is determining several ingredients in one natural product simultaneously. Vertical approach is determining one ingredient in several natural products by a single method. An example of determination of oleanolic acid in natural products using radix *Achyranthes bidentata* as a marker sample² was used as an example for elucidating vertical approach for the determination of ingredients in natural products.

Introduction

Recently, quality control for natural products becomes more and more important for ensuring medical efficacy and public safety. Natural products include all plants, animals and minerals. To evaluate the efficacy and toxicity of natural products, ingredients of natural products are required to be determined. However, the method of determination of ingredients were not standardized and optimized. The method for determination and the experimental results cannot be communicated and accumulated. Therefore, duplication and non-accumulation of works result. A huge amount of resources were wasted. A standardized method for determination is needed to avoid duplication of works in order to save time and resources. In addition, the works can be accumulated.

Techniques for determination

Determination of ingredients in natural products required the technique of high performance liquid chromatography (HPLC), electrochemical method, thin layer chromatography (TLC), gas chromatography (GC), capillary electrophoresis (CE), or inductively-coupled plasma – mass spectrometry. They are compared and summarized. After the comparison, choosing techniques for determination of ingredients in natural products depends on the nature of

ingredients and availability of instruments. HPLC was the most promising technique because of its high precision, high accuracy, low limit of detection, low limit of quantitation and suitability for wide variety of compounds³.

Methodology of determination

There are two main approaches:

Horizontal approach

Horizontal approach is the approach for determination of ingredients in natural products by developing HPLC fingerprint of a natural product and determination of more than one ingredient simultaneously. Simultaneous determination of ferulic acid, prim-O-glucosylecimifugin and isoferulic acid in *rhizoma cimicifugae* was studied as an example¹.

Vertical approach

Vertical approach is the approach for determination of ingredients in natural products by developing a standardized extraction procedures and HPLC method for determination of only one ingredient but the method can be applied to all natural products. Determination of oleanolic acid in natural products using radix *Achyranthes bidentatae* as a marker sample was studied as an example².

Post-determination

As the ingredients composed natural products are found and the amounts of ingredients in natural products are determined, the activities and toxicity of ingredients and natural products themselves can be evaluated. Oleanolic acid was taken as an example. There are 146 families, 698 genera and 1620 species of plants containing oleanolic acid up to Sep 2007 as reviewed³. The pharmaceutical activities and toxicity were also reviewed and it showed that Oleanolic acid has hepatoprotective, anti-stomach ulcer, hypoglycemic, anti-hyperlipidemic, anti-hypertensive, cardiogenic, anti-dysrhythmic, anti-aggregation of blood platelet, anti-cancer, protection of renal toxicity, anti-inflammatory, anti-mycobial and anti-fertility activities with low toxicity⁴. Radix *Achyranthes bidentatae*, an example of natural product, has analgesic, anti-inflammatory, blood circulation invigoration, stagnant blood clearing, anti-stomach ulcer, secretion of bile enhancement, anti-procreate and anti-implantation, blood glucose level reducing, lipoprotein reducing, protein assimilation increasing, anti-tumor, memory and endurance improvement, anti-aging, bone growth promotion, bone resorption inhibition, anti-asthmatic and hepatoprotective activities. It also enhances immune system by macrophages activation, increase in monocytes, activation of natural killer cells, suppress in spleenocyte, T-cells proliferation, induce secretion of IL-2, increasing C3bBb and ICR in immunosuppressed and normal mice peripheral blood, suppress in B-lymphocyte and immunoglobulin, with low toxicity as reviewed⁵. As

oleanolic acid is one of ingredient of radix *Achyranthes bidentatae* and shows activities of radix *Achyranthes bidentatae*, it is one of the active ingredients of radix *Achyranthes bidentatae*. Chromatographic fingerprint-activity relation can be developed after all ingredients of all natural products were determined and all activities of all ingredients were determined.

Conclusion

Methodology of determination of ingredients in natural products is important for quality control of natural products. A standardized method for determination is needed to avoid duplication of works and encourage accumulation of works for developing chromatographic fingerprint-activity relation of natural products.

References

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