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Application of immunoaffinity purification technology as the pretreatment technology for traditional Chinese medicine: Its application to analysis of hesperidin and narirutin in traditional Chinese medicine preparations containing *Citri reticulatae Pericarpium*

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ABSTRACT

In the present study, the feasibility of immunoaffinity chromatography (IAC) as a purification technology for the analysis of bioactive components in Traditional Chinese Medicine (TCM) was evaluated. IAC was used to analyze hesperidin (HP) and narirutin (NR) in TCM preparations containing *Citri reticulatae Pericarpium* (CRP, Chenpi in Chinese). An IAC column for the specific extraction and enrichment of HP and NR from TCM preparations containing CRP was developed and characterized. After HP reacted with carbonyl diimidazole and coupled to protein, it was used to immune mice for the generation of antibody. Through cell fusion, cloning and screening, monoclonal antibody was obtained. The IAC column was constructed by covalently coupling specific monoclonal antibody against HP and NR to CNBr-activated Sepharose 4B and packed into a common solid phase extraction cartridge. The extraction conditions including loading, washing and eluting, as well as flow rate for the extraction of HP and NR were optimized. Under optimal conditions, the maximum capacity, extraction recovery rate and stability of IAC column was also characterized. Results revealed that the maximum capacity of IAC column for HP and NR was approximately 16 μg and the relative binding capacity per 1 mL of the column volume was 27 μg. The extraction recovery rate of IAC column for HP and NR at three spiked levels was in the range of 94.05–109.15%. After the repeated application for 5 times, no significant loss of specific recognition was observed. Using high performance liquid chromatography (HPLC) as an effective analytic tool, HP and NR could be successfully separated via IAC column without the inference from impurities, suggesting that the extraction of HP and NR using the prepared IAC column is feasible. The application of IAC can solve the problem of quantitative analysis due to severe interference or low content. Furthermore, pretreatment methods in different matrixes can be unified. The IAC purification procedure can be used as an alternative effective analytical method for the pretreatment of bioactive components in TCM.

1. Introduction

Because of the complex components of Traditional Chinese Medicine (TCM) preparations and the low content of bioactive components, the successful analysis depends on the pretreatment of sample purification technology. With the development of science and technology, the detection technology and the efficiency of modern instrumental analysis greatly improved. However, the pretreatment methods for TCM preparations are still traditional, which results in a growing gap between the pretreatment of samples and detection technologies.

Traditional purification methods such as liquid–liquid extraction and column chromatography have multiple shortcomings including the requirement of large amount of organic solvents, time consuming and low selectivity. In addition, different methods need to be established according to different matrixes, resulting in more difficulties to unify the detection technology for different TCM preparations. Immunoaffinity chromatography (IAC), based on the highly specific interaction between antigen and antibody,

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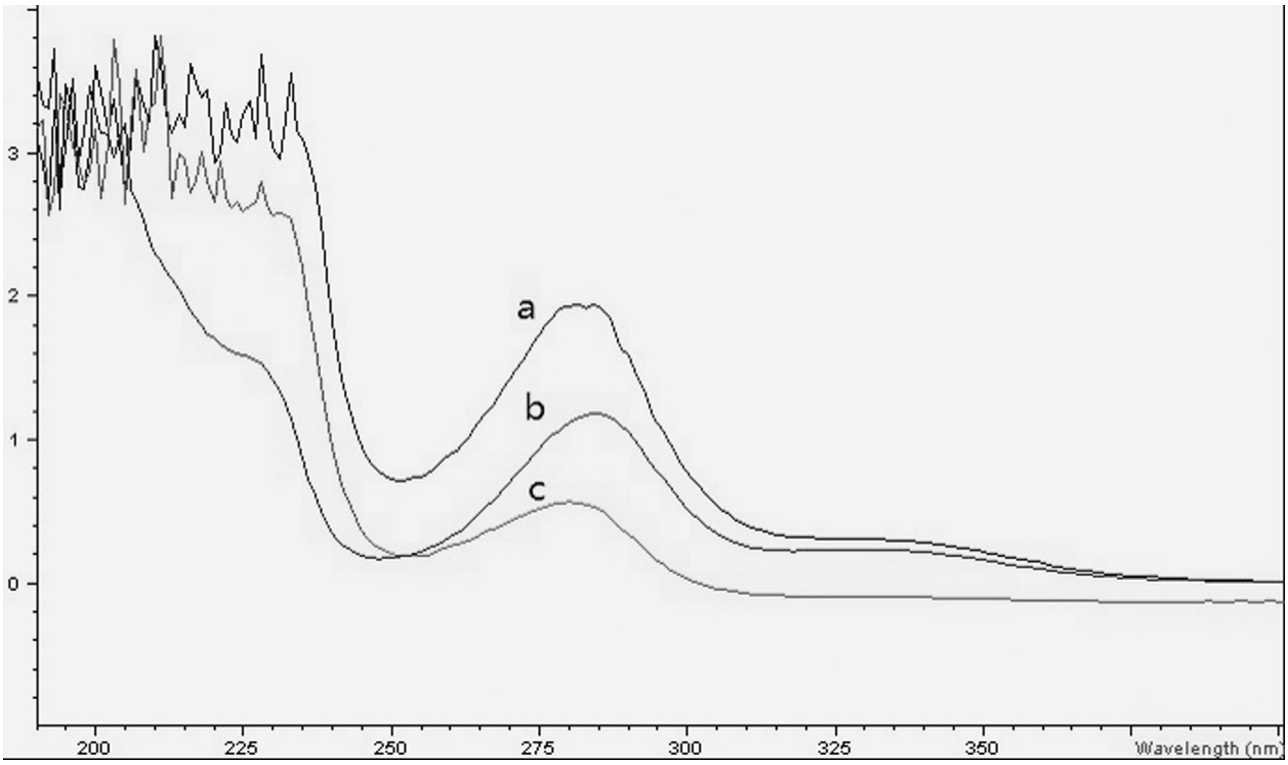

Fig. 1. UV spectra of hesperidin immunogen ((a) HP-BSA; (b) HP; (c) BSA).

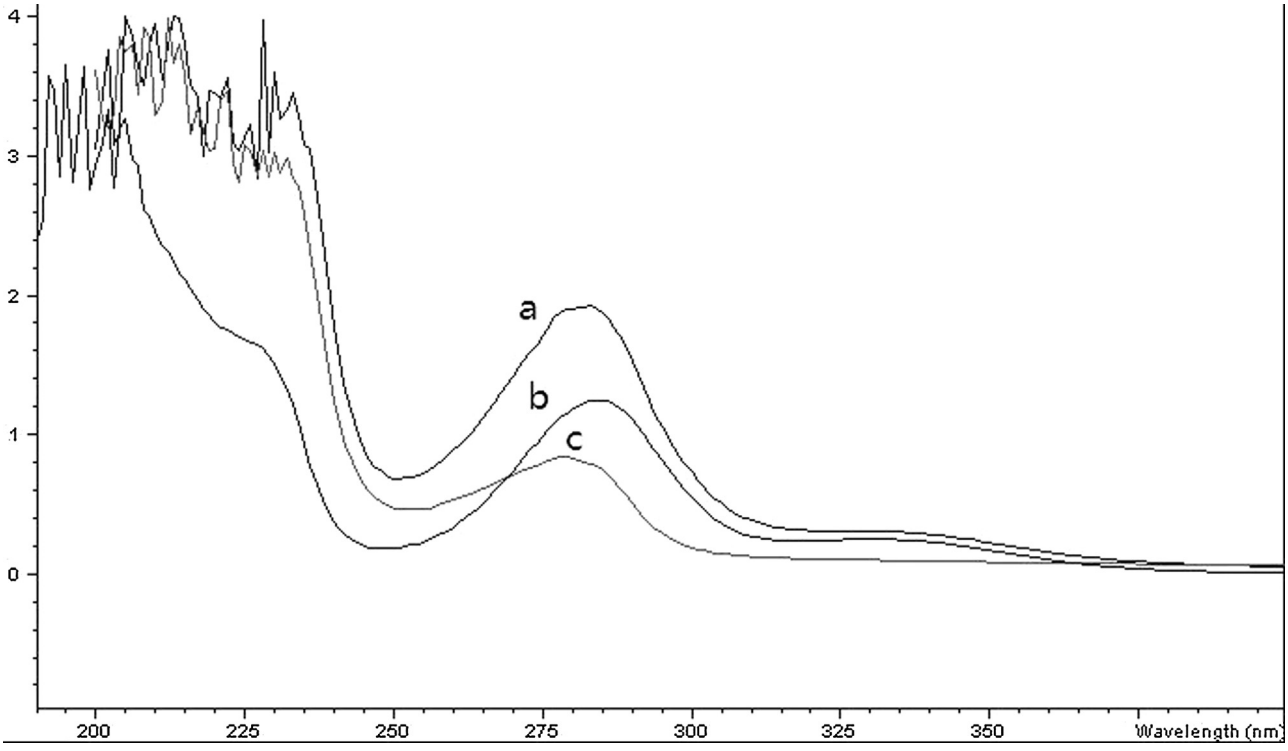

Fig. 2. UV spectra of hesperidin detection antigen ((a) HP-OVA; (b) HP; (c) OVA).

is an excellent method for the pretreatment of the samples. It has the advantage of the specific and reversible interaction between antibody and antigen, and has been considered as one of the most powerful techniques for single-step purification and condensation of the target analyte from complex matrixes [1,2]. Moreover, IAC method has the characteristics of highly effective purification, simple operation and environmental friendly due to the application of the relatively small amount of organic solvents in IAC. Because of high specificity, strong enrichment capability and purification efficiency in different matrixes of IAC, it has been widely used in many aspects. The application of IAC for the pretreatment of a variety of samples containing toxins, and biomacromolecule and drug