

# Preparation of high purity mulberry anthocyanin by preparative liquid chromatography



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### Introduction

Mulberry anthocyanins (MAY), which are commonly called mulberry red pigments, have numerous applications in the food industry. Due to its advantages of low cost, good pigment stability and colorability. due to the similar structure of anthocyanin,  $C_3G$  and  $C_3R$  are not able to be completed separated. Among several methods of anthocyanin monomer preparation, owing to its high sensitivity, economic feasibility and magnifiability, preparative liquid chromatography (PLC) has been widely used in the purification of various natural products.



### Content

In this work, to prepare high purity MAY efficiently, YWG C18 column (20 mm  $\times$  250 mm, 10  $\mu$ m) was used, column temperature was 30 °C, detection wavelength was 513 nm, and quantitative ring was 1 mL. The preparative chromatographic mobile phase was as follows: phase A was 4% acetic acid aqueous solution, and phase B was chromatographic grade methanol. A certain mass of mulberry red pigment freeze-dried powder was weighed, and a certain mass concentration of mulberry red pigment sample solution was prepared with 20% methanol, and filtered by 0.22  $\mu$ m organic filter membrane. Fig. 1 shows that when the flow rate is lower than 12 mL/min, the longitudinal molecular diffusion in the column plays a major role in the height of the tray, and the eddy current diffusion term plays a secondary role in the height of the tray. Fig. 2 reveals that region 1 and region 3 were selected as C3G and C3R monomer cutting collection region respectively, and the mixed component eluent of No. 2 region could be prepared twice after removing the mobile phase

#### Fig. 2 Fractional collection of peaks separated by semi-pre HPLC and product purity



Fig. 1 Conditions for Separation of Mulberry Red Pigment Monomers by Preparative High Performance Liquid Chromatography. (A) Preparative HPLC chromatogram of mulberry red pigment with different mobile phase gradient elution conditions; (B) Preparative HPLC chromatogram of mulberry red pigment with different flow.



Consequently, the new mulberry red pigment with high purity and high activity was obtained by preparative liquid chromatography, which opened up a new way for the comprehensive utilization of mulberry resources and further solved the market demand problem of high quality natural pigments.

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## References

[1] Agcam, E, et al. Food Chemistry, 237, 461-470.
[2] Wen,P, et al. Trends in Food Science & Technology, 83, 138-158..
[5] Zhou,T. B, et al. International Journal of Molecular Sciences, 12(5), 3006-3017.