Preparation of high purity mulberry anthocyanin by preparative liquid chromatography

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In China, mulberry is widely planted and is one of the most abundant dietary sources of polyphenols and anthocyanins compared with other berries. Mulberry red pigment is natural colorants which have aroused a growing interest due to bright color, innocuous and beneficial health effects (Agcam et al., 2017). Due to mulberry containing active substances that promote human health, it has been used as a traditional medicinal food material for the treatment of various diseases (Wen et al., 2019). Equally, mulberry anthocyanin has a variety of biological activities such as antibacterial, anti-inflammatory and antioxidant, as the main bioactive components of mulberry (Zou et al., 2011). However, the purity of mulberry pigments in the current market is generally low, which does not meet the market demand in terms of economic benefits and health needs. Therefore, it is necessary to find an efficient separation and purification method to prepare high purity mulberry pigment.

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 (CastañedaOvando et al., 2009). Moreover, MAY have drawn increasing attentions owing to their potential roles of natural antioxidants. The primary anthocyanins identified in mulberry are cyanidin 3-O-rutinoside (C3R), cyanidin 3-O-glucoside (C3G) and other elements. C3G, accounting for about 60% of the total, is the main coloring component and has potent antioxidant and anti-inflammatory effects. C3R (about 30%) exhibits high antioxidant activities (Natić et al., 2015), whereas it was rarely used in the food matrix. Due to the low anthocyanin content, various components and polarity differences in raw materials, the preparation technology of anthocyanin is limited. In addition, due to the similar structure of anthocyanin, C3G and C3R 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.

In this work, to prepare high purity MAY efficiently, YWG C18 column (20 mm×250 mm, 10 μm) 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 μm organic filter membrane.

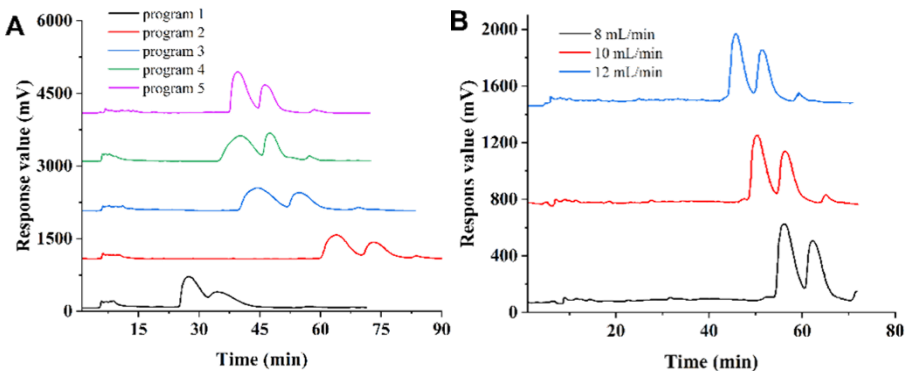

Fig. 1 Conditions for Separation of Mulberry Red Pigment Monomers by Preparative High Performance Liquid

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 rates;

Fig. 1 indicates that with gradient elution program 5, the peak shape is symmetrical and the peak width becomes narrow, which is more conducive to the concentrated elution and concentration recovery of the target substance.. 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.

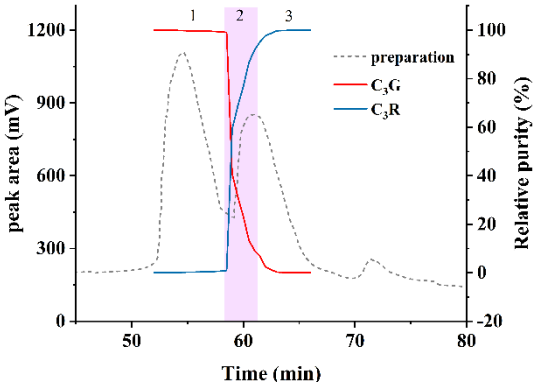

Fig. 2 Fractional collection of peaks separated by semi-pre HPLC and product purity

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.

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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