

P99. Fast HPLC method for anthocyanins determination in pomegranate (*Punica granatum* L.) juice

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Pomegranate juice has a high antioxidant activity, due especially to its anthocyanins content (delphinidin 3,5-diglucoside; cyanidin 3,5 diglucoside; delphinidin 3-glucoside; pelargonidin 3,5-diglucoside; cyanidin 3-glucoside; pelargonidin 3-glucoside). Furthermore, the quantification of these compounds in pomegranate juice is still scarce. The objective of this work was to show a faster High performance liquid chromatography (HPLC) method for anthocyanins determination in pomegranate juice, using a new technology particles column. HPLC methods generally use C18 columns (150 x 4.6mm; 3.5µm) resulting in a long run sample. The demand for cleaner, faster and more economical and efficient methods is increasing in the last times. The development of new technologies used for the preparation of stationary phase with smaller diameter particles, made possible new alternatives to reduce the run time, without compromising chromatographic separation. Initially, the chromatography method was performed on a Waters® Alliance 2695 system, with a Waters® 2996 photodiode array detector, with a conventional C18 column (150 x 4.6mm; 3.5µm), flow 1.0 mL/min., column temperature of 30°C, injection volume of 50µL and gradient elution method with methanol and formic acid. At the faster method tested, besides the different column - Thermo® Scientific C18 BDS (100mm x 4.6mm; 2.4µm), the column temperature used was 40°C, with flow 1.0 mL/min., injection volume of 20µL and gradient elution method with acetonitrile and formic acid. With the new method, run time changed from 35 minutes to 14 minutes, representing a solvent saving of 65%. It was possible to obtain resolution of 100% for four peaks, and 96% for the cyanidin 3,5-diglucoside in relation to the delphinidin 3-glucoside, which is also considered a satisfactory value to have a good integration of the peaks. Besides that, with these new conditions, were obtained thinner peaks, which reduce the chances of having co elution of them.