INVESTIGATION OF HIGHSPEED COUNTERCURRENT CHROMATOGRAPHY (CCC) FOR THE SEPARATION OF TEA CATECHINS

N. S. KUMAR AND K.T.P. M. P. RAJAPAKSHA

Department of Chemistry, Faculty of Science, University of Peradeniya

High speed counter current chromatography (HSCCC) is a liquid-liquid separation method that does not use a solid support matrix and which permits total recovery of the sample. Decomposition is minimised and the technique is important in the separation of unstable compounds and highly polar compounds. During the separation it is possible to reverse the direction of flow and commence elution of compounds retained in the stationary phase. The use of centrifugal force allows an increase in the speed of separation without loss of resolution. HSCCC is a low cost alternative for preparative isolation and separation processes.

Catechins were extracted from a commercial sample of green tea leaves (100 g) by boiling with water followed by extraction with EtOH. The combined extracts were concentrated under reduced pressure to 1/3 volume and extracted with an equal volume of $CHCl_3$ to remove caffeine and other pigments. The catechins were extracted from the aqueous layer using EtOAc, evaporated to dryness, dissolved in water, and freeze dried to give the catechin mixture (3.5 g).

HSCCC requires the use of a suitable two phase solvent system. It has been recorded that the partition coefficient (K) of the two phase solvent system should be between 0.5-1.5 (K=Cs/Cm where Cs = concentration in the stationery phase and Cm = concentration in the mobile phase) K values were determined using a UV spectrophotometer (λ_{max} 306.5 nm). It was found that K should be between 0.9-1.3 for efficient retention of the catechin mixture in the mobile phase. A two phase solvent system composed of hexane: ethyl acetate: methanol: water 1.5:10:1.5:10, (K of 1.2) was used for the separation of tea catechins. The solvent mixture was prepared in a separatory funnel and the two phases were separated shortly before use. The coil column was filled entirely with the stationary phase and the mobile phase was then pumped in with a flow rate of 1.5 ml/min. The most effective rotation speed for the coil was found to be 800 rpm. After the two phases achieved hydrodynamic equlibrium, the sample (150 mg) dissolved in the mobile phase (5 ml), was introduced through the injection valve.

The catechin mixture was separated into fractions A, B, C, D and E from the mobile phase and fractions F and G from the stationery phase. Fractions A and B contained a single catechin each. Fraction F did not contain catechins, and was a mixture of three UV active compounds. Fraction G contained a single catechin. Our results indicate that the method is suitable to separate mixtures of catechins.

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