

## FRACTIONATION OF GREEN TEA CONSTITUENTS USING HIGH SPEED COUNTERCURRENT CHROMATOGRAPHY (HSCCC)

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

*Department of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya.*

Catechins are flavonoids that constitute up to 30% of the dry weight of young tea shoots. It has been suggested that tea catechins may be responsible for the health benefits of green tea. Pure samples of catechins are necessary to conduct further studies regarding the role of individual catechins in reducing the risk of certain types of cancer and cardiovascular disease. This paper is a report of a study carried out on the use of High Speed Countercurrent Chromatography (HSCCC) to fractionate green tea extracts, in order to isolate pure samples of tea catechins.

The catechin mixtures were extracted from fresh young vegetative shoots of five different tea clones, TRI 2023, TRI 2025, TRI 2043, TRI 3079 and TRI 4006, using aqueous 70% methanol, and then fractionated using HSCCC. The crude catechin extract was partitioned in solvent mixtures containing different ratios of hexane, ethyl acetate, methanol and water. The partition coefficient ( $K$ ) was calculated by measuring the absorbance at  $\lambda_{\text{max}}$  280 nm where  $K = C_s/C_m$  ( $C_s$  = concentration of solute in the stationary phase, and  $C_m$  = concentration of solute in the mobile phase). These calculations were performed by selecting the lower phase as the mobile phase and the upper phase as the stationary phase. In each fractionation, the solvent system which gave  $K \approx 1$  was selected. The fractions thus obtained were analyzed by HPLC.

The purity of each epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and theobromine (TB) samples obtained was more than 90%. Two other unidentified compounds, FLA1 and FLA2 (> 90%), were also isolated in high purity, from the tea clones, TRI 4006, TRI 2023 and TRI 2025. Pure samples of epigallocatechin (EGC) were obtained from the tea clones, TRI 2023 and 4006, while pure epicatechin (EC) was isolated from TRI 2025.

*The authors thank Sida (SAREC) and IPICS for financial support.*

