DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF VITAMIN C AND CITRIC ACID IN BEVERAGE PREMIXES

Hewapathirana J.C[1*], Malvipathirana S[1] and Perera P.S.F[2]
[1]Department of Physical Sciences &amp Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Sri Lanka
[2]Chemical & Microbiological Laboratory, Industrial Technology Institute, Sri Lanka.
[*]jchathuranga2012@gmail.com

Vitamins play an important role like other basic nutritional components. Vitamin C is among the essential vitamins, which not synthesized in the human body, which must be supplied through a balanced daily diet. Two of them such common additives, especially in the food industry, are vitamin C and citric acid. Vitamin C acts as an antioxidant and citric acid balances the taste and prevents browning reactions. Confirmation of enriched and reported levels of these additives in various foods is an imperative requirement for QA / QC aspects of foods. This research conducted to validate a concurrent assay method for vitamin C (*L*-ascorbic acid) and citric acid in foods and beverages with reverse- phase liquid chromatography (RP-HPLC). The analysis was carried out under isocratic mobile phase conditions using 250 mm $\times$ 4.6 mm, 5 μm C_{18} column as follows, 20 mmol L^{-1} monobasic phosphate buffer (A) and a mixture of methanol and acetonitrile in 60:40 as mobile phase B were used in 75:25 ratio. Detection was carried out using UV-DAD detector at the wavelength of 230 nm with 7.00 min runtime. Validation of the method performed under terms of the Eurachem guidelines. The detection limits for vitamin C and citric acid were 0.5μg mL^{-1} and 20 μg mL^{-1} , respectively. The linearity indicated by the regression value (R^2) for vitamin C was 0.9999 at the working rage of 0.5 mg kg^{-1} to 500 mg kg^{-1} and that for citric acid was 0.9998 for the working range of 20 mg kg^{-1} to 600 mg kg^{-1} . The recovery of the method was between 80% and 120% for both vitamin C and citric acid. RP-HPLC was found to be a reliable detection method for vitamin C and citric acid simultaneously in the foods and beverages.

Keywords: *RP-HPLC method, Vitamins C, Citric acid, Simultaneously determination, Beverages*