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Validation of RP-HPLC method to quantify vitamin C and citric acid in ready to serve fruit juice – A simultaneous approach

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A balanced diet is an imperative need according to the recommended daily allowances for a healthy life. Vitamins play a big role, as do the other main nutritional components – carbohydrates, protein, fat, fiber and essential minerals. Vitamin C, one of the important vitamins not synthesized in the human body, needs to be supplied via the diet in sufficient quantities. Fortification of food with such ingredients is a common practice. Two such common additives, specially in food industries, are vitamin C and citric acid. Vitamin C acts as an antioxidant and citric acid enhances the flavour and prevents the browning reactions. Assurance of the levels fortified and stated in labels of such auxiliary materials in diverse foods is an imperative requirement of food QA/QC aspects. Despite individual methods used for vitamin C and citric acid determinations, this study attended to validate a concurrent method for the determination of vitamin C (*L-ascorbic acid*) and citric acid in fruit juices with reverse phase high performance liquid chromatography (RP-HPLC) subsequent to optimization of analytical parameters common to both analytes. Analyses were done using isocratic conditions of 75: 25, where mobile phase A was 20 mM phosphate buffer, while B was a mixed mobile phase of methanol and acetonitrile. Detection was carried out at the wavelength of 230 nm. Validation of the method was carried out against accuracy, precision, reproducibility, linearity range, minimum detectable level, and recovery, as per the Eurachem guidelines. The detection limits for vitamin C (ascorbic acid) and citric acid were 0.5 µg/mL and 20 µg/mL, respectively. The linearity given by regression value (R^2) for vitamin C was 0.9999 at the working range of 0.5 mg/kg to 500 mg/kg and that of citric acid was 0.9998 for the working range of 20 mg/kg to 600 mg/kg. The recovery of the method was within the range of 80% to 120% for both vitamin C and citric acid. The accuracy of the method was established by using QC samples, which fall in the accuracy range. As per the results, the method provides a reliable way to detect both vitamin C and citric acid simultaneously, in ready to serve fruit juices as well as beverages in the market. The method thus provides an analytical protocol for QA/QC and service providing laboratories in the respective area. The validated method can be effectively and simultaneously used to quantify vitamin C and citric acid.

Key words: Citric acid, Eurachem guidelines, RP-HPLC, method validation, vitamins.

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