

Validation of a reversed-phase high performance liquid chromatographic method for the determination of stevioside and rebaudioside A in food and beverages

P.S.F. Perera^{1*}, H.P.P.S. Somasiri¹, K.R.R. Mahanama²

¹Industrial Technology Institute, Colombo 7, Sri Lanka.

²Department of Chemistry, University of Colombo, Sri Lanka.

Currently there is a trend of using non-caloric high-intense sweeteners, as substitutes for sugars in food, carbonated and non-carbonated beverages to acquire the required sweet taste. Steviol or steviol glycosides that has stevioside followed by rebaudioside A, as major components is used as one of non-caloric natural sweeteners with a sweetening potential of 250-300 times more than that of sucrose. The aim of the present study is to develop and validate a HPLC method to determine the most abundant steviol glycosides in Sri Lankan food and beverages. The analytes were separated by C18 column (250 mm x 4.5 mm, 5 μ m) at 35 °C with an eluent composed of 20 mM phosphate buffer and acetonitrile in a ratio of 65:35 (v/v). Detection was carried out with the diode array detector at the wavelength of 210 nm. Validation of the method performed in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, working range, precision and accuracy according to the Eurachem guidelines using USP reference standard. LOD values for stevioside and rebaudioside A, were 2.6 mg/kg and 3.9 mg/kg while the LOQ values were 3.1 mg/kg and 4.6 mg/kg respectively. Working range of stevioside and rebaudioside A were 2 mg/kg to 500 mg/kg and 5 mg/kg to 500 mg/kg respectively with a linearity of ≥ 0.9999 . Accuracy for stevioside was between 86.8%-102.4% while for rebaudioside A, it was between 80.7%-118.7% for spiked levels from 25 mg/kg to 300 mg/kg. The precision for both stevioside and for rebaudioside A, were lower than 3.5% when expressed as relative standard deviation. In conclusion, the developed analytical procedure is sensitive, simple, and accurate to determine the levels of stevioside and rebaudioside A, in carbonated and non-carbonated beverages and sugar free biscuits.

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*sujitha@iti.lk