INTISARI PENELITIAN

Stevia rebaudiana mengandung senyawa aktif steviosida dan rebaudiosida A yang berpotensi sebagai kandidat obat antidiabetes tipe 2 dan telah dimanfaatkan untuk pemanis alami. Tujuan penelitian ini adalah mengembangkan metode analisis RP-HPLC isokratik beserta validasi metode dan aplikasinya untuk penetapan kadar kedua analit yang dituju dalam ekstrak etanolik daun *S. rebaudiana* dan produk simulasi minuman, mengembangkan metode preparasi sampel secara *Solid Phase Extraction* (SPE) baik fase terbalik maupun fase normal untuk analisis HPLC, melakukan studi degradasi senyawa analit secara hidrolisis, pemanasan kering dan paparan $UV_{254\ nm}$, dan mengembangkan metode analisis spektrofotometri FTIR yang dikombinasi dengan kalibrasi multivariat PLS untuk penetapan kadar steviosida dan rebaudiosida A dalam ekstrak etanolik kering daun *S. rebaudiana* dan produk simulasi minuman Stevia. Hasil penelitian menunjukkan kondisi optimal HPLC yang dikembangkan adalah fase diam Eurosphere C-18 (250 × 4,6 mm., i.d., 5 µm) pada suhu 30^{o}C, fase gerak campuran akuades : metanol (90 : 10, v/v disesuaikan pH 3,00 dengan asam fosfat 0,1%) (pelarut A) dan asetonitril (pelarut B) dengan komposisi pelarut A : B = 65 : 35 (v/v) dan ditambahkan asam trifluoroasetat 0,01% (v/v), kecepatan alir 0,6 mL/min, detektor UV pada $\lambda_{210\ nm}$. Metode HPLC yang dikembangkan dapat memenuhi jaminan validasi metode dan berhasil diaplikasikan untuk penetapan kadar steviosida dan rebaudiosida A dalam sampel. Metode preparasi sampel SPE yang dikembangkan memenuhi jaminan akurasi dan presisi. Metode NP-SPE (silika) memiliki mekanisme *selective elution* sedangkan mekanisme metode RP-SPE (C-18) adalah *selective washing*. Degradasi steviosida dan rebaudiosida A ditunjukkan dengan terputusnya ikatan glikosida kedua analit. Metode RP-HPLC yang dikembangkan memiliki selektivitas yang baik (Rs > 2,0) terhadap senyawa hasil degradasi yang terdekat. Berdasarkan uji *equivalence pair t test*, metode analisis kuantitatif spektrofotometri FTIR yang dikombinasi dengan analisis multivariat PLS memberikan nilai prediksi yang *equivalence* dengan nilai aktual metode referens HPLC untuk penetapan kadar kedua analit dalam sampel.

Kata kunci: RP-HPLC isokratik, FTIR, steviosida, rebaudiosida A, *Stevia rebaudiana*

ABSTRACT

Stevia rebaudiana leaf contain active compounds of stevioside and rebaudioside A which is potentially as type 2 antidiabetic drug candidate and has been used for a natural sweetener. The purposes of research were to develop isocratic RP-HPLC analysis method and to apply method developed for determining stevioside and rebaudioside A content in *S. rebaudiana* leaves and Stevia beverages simulation product, to develop Solid Phase Extraction (SPE) method, both of reverse (RP-SPE) (C-18) and normal phase (NP-SPE) (silica) followed by RP-HPLC analysis application, to conduct degradation study of stevioside and rebaudioside A, particularly using stressor of hydrolysis, thermal and UV $_{254\ nm}$ radiation, and to establish quantitative FTIR spectroscopy analysis method in combination with multivariate analysis using PLS regression to determine stevioside and rebaudioside A content in dried ethanol extract of *S. rebaudiana* leaf and Stevia beverages simulation product. The results showed that isocratic RP-HPLC analysis method was conducted on Eurosphere C-18 (250 × 4.6 mm, i.d., 5 µm) as stationary phase. Mobile phase was made by a mixture of water-methanol (90: 10, v / v, pH = 3.0) (solvent A) and acetonitrile (solvent B), in the ratio of A : B = 65: 35 (v/v) and was added by trifluoro acetic acid (TFA) of 0,01% (v/v) in to the mixture. Flow rate of mobile phase applied was 0.6 mL/min. The detection was performed in UV-210 nm. The method developed met completely in method validation and was successfully applied to determine stevioside and rebaudioside A content in sample. SPE methods developed achieve accuracy and precision assay. NP-SPE (silica) has selective elution mechanism to elute the analytes while RP-SPE (C-18) has selective washing mechanism. Stevioside and rebaudioside A degradation was performed by cleavage of glycoside bond. Isocratic RP-HPLC method developed still has good resolution (Rs > 2,00) of the analyte from nearest degradation compound. Based on equivalence pair t test analysis, quantitative FTIR spectroscopy method in combination with multivariate analysis of PLS was equivalence with reference method of HPLC to determine stevioside and rebaudioside A content in sample.

Keywords: Isocratic RP-HPLC, FTIR, stevioside, rebaudioside, *Stevia rebaudiana*