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DETECTION OF COUNTERFEIT STEVIA PRODUCTS USING HPLC-ELSD

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ABSTRACT

Diabetes prevalence in Indonesia has been increasing every year. Indonesia becomes the world's 5th largest country with diabetic population, estimated to be about 10% of the population and about 51% of those were undiagnosed. People with diabetes mellitus are required to avoid sugar (sucrose) in their daily meals and replace it with safe sugar substitutes. Stevia from *Stevia rebaudiana Bertoni* is known as natural sugar substitutes which safe for people with diabetes melitus. *Stevia rebaudiana* is also traditionally used to treat diabetes mellitus. The standard mixtures of Stevioside, Rebaudioside A, Rebaudioside C and Dulcoside A were injected into HPLC-ELSD under optimum condition to obtain the chromatograms. Samples of stevia products were extracted with methanol, and methanol extract was injected into HPLC-ELSD under the same conditions as standard solution and chromatograms were obtained. Chromatograms from stevia product samples were compared with chromatograms from Stevioside standard solutions, Rebaudioside A, Rebaudioside C and Dulcoside A. Based on the results and the above discussion it can be concluded that from the five samples, samples 1 and 2 contain stevia sweetener, sample 3 contains no stevia sweetener but rebaudioside A. Sample 4 and 5 contain neither stevia nor steviol glycoside sweeteners.

KEYWORDS: Stevia, Counterfeit, HPLC-ELSD, Stevioside, Rebaudioside, Dulcoside.

INTRODUCTION

Diabetes prevalence in Indonesia has been increasing every year. Indonesia becomes the world's 5th largest country with diabetic population, estimated to be about 10% of the population and about 51% of those were undiagnosed (AFES 2017). People with diabetes mellitus are required to avoid sugar (sucrose) in their daily meals and replace it with safe sugar substitutes. Stevia from *Stevia rebaudiana Bertoni* is known as natural sugar substitutes which safe for people with diabetes melitus (Supriyadi *et al*., 2016). *Stevia rebaudiana* is also traditionally used to treat diabetes mellitus. The use of *Stevia rebaudiana* leaves as a substitute for sugar, does not raise blood sugar levels and does not cause obesity (Brahmachari *et al*., 2011). Stevia can also help to lose weight (Elnaga *et al*., 2016).

The sweet taste of *Stevia rebaudiana* is caused by several steviol glycoside compounds, including stevioside (±13.955%), rebaudiosida A (± 1%), rebaudiosida C (±0.5%), and dulcoside A (± 0.56%) (Supriyadi *et al*., 2016). Stevia has 30 times sweeter taste than sugar (sucrose), while pure stevioside has 200 times sweeter taste than sucrose (Raini and Isnawati, 2011).

According to WHO (2006) the Acceptable Daily Intake (ADI) for steviol glycosides from *Stevia rebaudiana* is 4 mg / kg BW / day. The recommended maximum dose in Japan is 3 mg / kg BW / day and in USA 5 mg / kg BW / day. At that dose, stevia is safely consumed as a sugar substitute and non-calorie sweetener (Shanon, 2016). According to the Food Drug and Administration (FDA) (2008), Stevia is a safe product for consumption up to 1500 mg per day, but the FDA does not recommend stevia used for pregnant and lactating women. WHO (2006) concluded that stevioside and rebaudioside are not mutagenic either in vitro or in vivo. WHO also reported that stevioside, rebaudioside and steviol are not carcinogenic. Stevioside also provides pharmacological effects in patients as antihypertensive and antidiabetic (Benford *et al*., 2006; Chatsudthipong & Muanprasat, 2009). Stevioside is stable at high temperatures unlike saccharin and aspartame, the compound holds on heating up to 200^{o}C, so it can be used as a sweetener on almost all foods (Raini and Isnawati, 2011).

Steviol glycosides are secondary metabolites, these compounds are glycoside tertpenes consisting of steviol aglycone and sugar molecules. Steviol glycosides is the main stevia rebaudiana (Stevioside, Rebaudioside A, Rebaudioside C and Dulcoside A) (Supriyadi *et al*., 2016). Stevia products are generally obtained from the

Stevia rebaudiana plant through extraction and purification (Purkayastha *et al*., 2016). Nowdays, sweetener products from *Stevia rebaudiana* can be found in the form of powder, solution in sachets and in the form of tea bags. The sachet products contain *Stevia rebaudiana* extract and other additional ingredients, while the tea bag product is a blended form of tea leaf powder and *Stevia rebaudiana* leaf powder.

The use of *Stevia rebaudiana* extract in Indonesia has been legalized by Badan Pengawas Obat dan Makanan (BPOM) with Decree of the Head of POM Agency no. HK.00.05.52.3877 Year 2004 and has been updated with Decree of the Head of POM No. 4 of 2014, regarding Terms of Use Stevia Extracts as Natural Sweetener. Quality assurance of *Stevia rebaudiana* products is an important aspect to ensure the quality of products circulating in the market as well as in compliance with consumer protection laws. Quality assurance aims to ensure that the products are safe to use by consumers and have a composition in accordance with their etiquette. Steviol glycosides in *Stevia rebaudiana* are stevioside, rebaudioside A, rebaudioside C and dulkoside A. If there is a product with stevia etiquette claim using stevia sweetener, the product should contain stevioside, rebaudiosida A, rebaudiosida C and dulcoside A, otherwise the product can be included as a counterfeit one. Currently, as in the market there are many products with stevia etiquette informing that they use stevia sweeteners, it is necessary to detect the presence of stevioside, rebaudioside A, rebaudioside C and dulkoside A on them. Detection of counterfeit stevia products using raman spectrometer revealed that several samples of stevia products did not contain stevia sweeteners (Jentzsch *et al*., 2016).

Detection on counterfeit stevia products using HPLC-ELSD is done by comparing HPLC-ELSD chromatograms from the extracts of stevia products with HPLC-ELSD chromatograms on standard mixtures of stevioside, rebaudioside A, rebaudioside C and dulcoside A. If the stevia product chromatograms have similar chromatograms of stevioside standard solution, rebaudioside A, rebaudioside C and dulcoside A, then the stevia products properly contain stevia sweeteners (Supriyadi *et al*., 2016).

MATERIALS AND METHODS

Standard of Stevioside, Rebaudiosida A, Rebaudioside C and Dulkoside A (Sigma-Aldrich), Methanol and acetonitrile (E-Merck), and stevia products, were purchased from the market in Surakarta. HPLC-ELSD was used for the separation and detection of Stevioside, Rebaudioside A, Rebaudioside C and Dulkoside A mixtures, in this study. The ELSD detector is capable for detecting either the compound having no chromophores or having a weak chromophore such as Stevioside, Rebaudioside A, Rebaudioside C and Dulkoside A. To get optimum HPLC-ELSD conditions for mixed analysis of Stevioside, Rebaudioside A, Rebaudioside C and Dulkoside A, Luna Phenomenex phase acetonitrile motion column: water = 35: 65 with flow rate 1 ml / min, column temperature 50^{o}C , nebulatory temperature and detector temperature of 40 and 50^{o}C detectors are conducted. (Supriyadi *et al*., 2016).

The standard mixtures of Stevioside, Rebaudioside A, Rebaudioside C and Dulcoside A were injected into HPLC-ELSD under optimum condition to obtain the chromatograms. Samples of stevia products were extracted with methanol, and methanol extract was injected into HPLC-ELSD under the same conditions as standard solution and chromatograms were obtained. Chromatograms from stevia product samples were compared with chromatograms from Stevioside standard solutions, Rebaudioside A, Rebaudioside C and Dulcoside A.

Chromatograms of standard Steviocide, Rebaudioside A, Rebaudioside C and Dulcoside A and Chromatogram samples can be seen from figure 1 to 5 as follows:

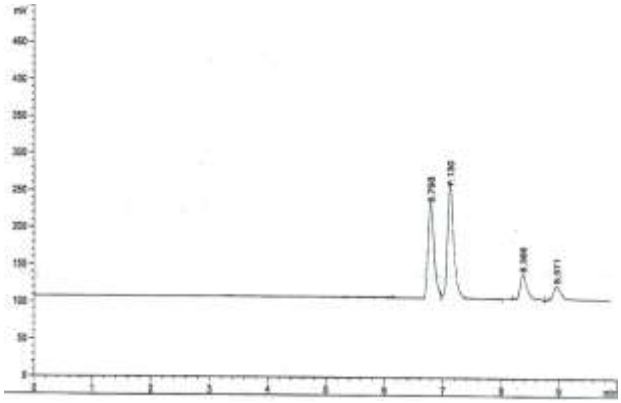

Fig. 1: Chromatogram of standard solution mixture Rebaudioside A, Steviosida, Rebaudioside C, and Dulcoside A.

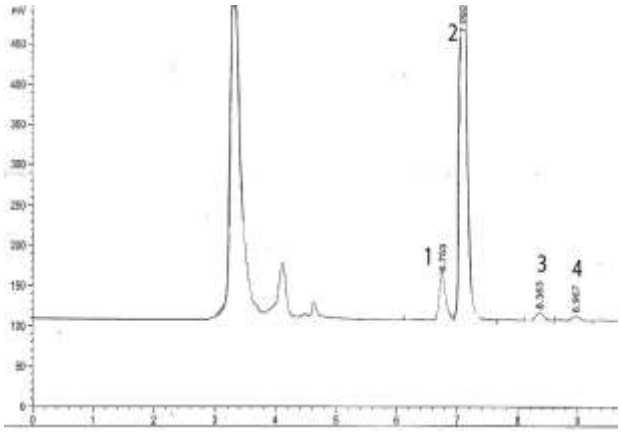

Fig. 2: Sample Chromatogram 1.

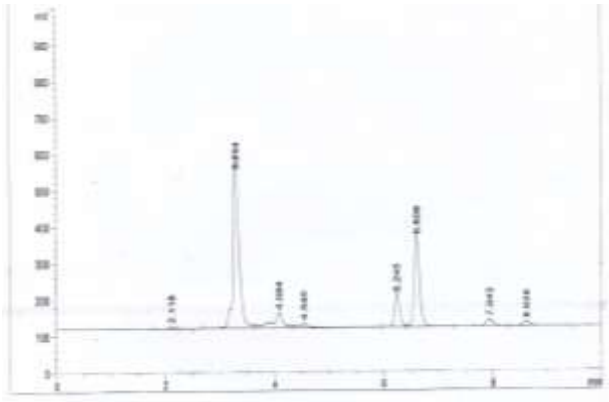

Fig. 3: Sample Chromatogram 2.

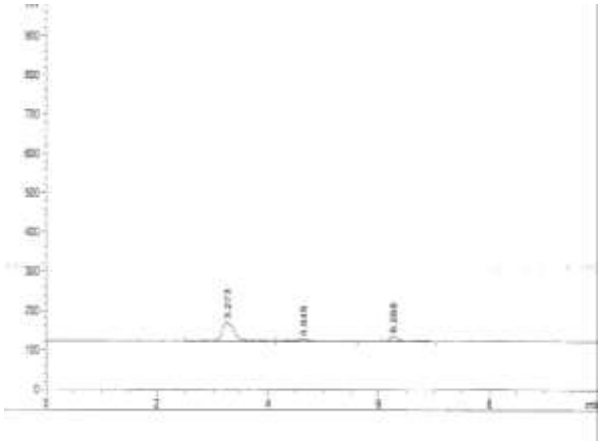

Fig. 4: Sample Chromatogram 3.

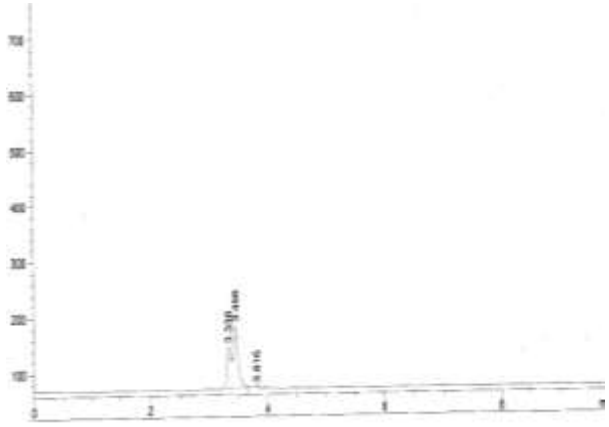

Fig. 5: Sample Chromatogram 4.

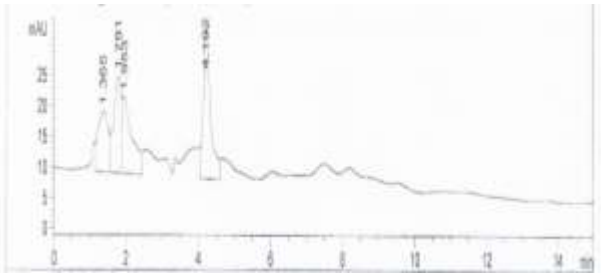

Fig. 6: Sample Chromatogram 5.

Chromatogram shown in figure 1 is a chromatogram of a standard solution mixture, the peak with retention time of 6.798 min is Rebaudioside A, 7.130 minutes Stevioside, 8.386 min is Rebaudioside C and 8.971 min is Dulkoside A.

Chromatogram shown in figure 2 is sample 1, which has peaks with retention times of 6.753, 7.992, 8.363 and 8.967 minutes similar to chromatogram patterns of standard solution mixtures Rebaudioside A, Stevioside, Rebaudioside C and Dulcoside A, which the main steviol glycosides present in stevia. This suggests that sample 1 contains stevia sweeteners because they contain the major steviol glycosides present in stevia.

Chromatogram shown in figure 3 is sample 2 which has peaks with retention times of 6.245, 6.608, 7.943 and 8.628 min similar to standard solution mixed chromatograms but are shifted to smaller retention times because the sample is more acidic than the mixed-solution standard, it shows that sample 2 also contains stevia sweetener.

Chromatogram shown in figure 4 is sample 3, there are several peaks, but there is only 1 peak at 6.268 minutes which is identical to the peak of standard solution rebaudioside A. This suggest that sample 3 only contains sweetener rebaudioside A instead of stevia sweetener.

Chromatogram shown in figure 5 is sample 4, there are several peaks, but no peaks which similar to chromatogram profile of the standard solution, therefore samples 4 do not contain stevia sweeteners.

Chromatogram shown in figure 6 is sample 5. There are several peaks, but no peaks which similar to chromatogram profile of the standard solution, therefore samples 4 do not contain stevia sweeteners.

CONCLUSION

Based on the results and the above discussion it can be concluded that from the five samples, samples 1 and 2 contain stevia sweetener, sample 3 contains no stevia sweetener but rebaudioside A. Sample 4 and 5 contain neither stevia nor steviol glycoside sweeteners.

REFERENCE

1. Badan Pengawas Obat dan Makanan (BPOM). *SK Kepala Badan POM No. HK.00.05.52.3877 Tahun 2014, Tentang Persyaratan Penggunaan Ekstrak Stevia Sebagai Pemanais Alami.* Jakarta, Pengawas Obat dan Makanan, 2014; 1-4.
2. Benford, D.J., DiNovi, M. and Schlatter, J. Safety evaluation of certain food additives: Steviol Glycosides. *WHO food additives series,* 2006; 5: 117-144.
3. Brahmachari, G., Mandal L.C., Roy, R., Mondal, S., and Arun, K.B. Stevioside and related compounds – molecules of pharmaceutical promise: a critical

overview. *Arch. Pharmceutical Chemstry Life Sciences*, 2011; 1: 5–19.
4. Chatsudthipong, C. & Muanprasat, C. Stevioside and related compounds: therapeutic benefits beyond sweetness. *Pharmacology & Therapeutics*, 2009; 121: 41–54.
5. Chaturvedula, V.S.P. and Zamora, J. Reversed-Phase HPLC Analysis of Steviol Glycosides Isolated from Stevia rebaudiana Bertoni. *Food and Nutrition Sciences*, 2014; 5: 1711-1716.
6. Elnaga, N.I.E.A., Massoud, M.I., Yousef, M.I. and Mohamed, H.H.A. Effect of stevia sweetener consumption as non-caloric sweetening on body weight gain and biochemical's parameters in overweight female rats. *Annals of Agricultural Science*, 2016; 30: 21-30.
7. Gengaihi, El., Souad, Nabaweya, I., Riad, Sahar, Regal, El.S.N., and Naglaa, S Sweet ent-kaurene diterpene glycosides of Stevia rebaudiana Leaves Bertoni and Biological Evaluation, *Journal of American Science, Biochem*, 2011; 7(6): 775-782.
8. Jentzsch, P.V., Vallejos, S.T., Brito, S.M., Luis A. Ramos, L.A. and Ciobota, V. Detection of counterfeit stevia products using a handheld Raman spectrometer, *Vibrational Spectroscopy*, 2016; 83: 126-131.
9. Kongres AFES 2017, Masalah Diabetes Jadi Bahasan Utama, http://www.berita satu.com/ kesra/150064-masalah-diabetes-jadi-bahasan-utama-di-kongres-afes-2017.html.Kamis 14 November 2017.
10. Konoshima, T. and Takasaki, M. Cancer-chemopreve, 2016; 77: 125-133.
11. Purkayastha, S., Markosyan, A., Prakash, I., Bhusari, S., Pugh Jr, G., Lynch, B. and Roberts, A., Steviol glycosides in purified stevia leaf extract sharing the same metabolic fate. *Regulatory Toxicology and Pharmacology*, 2016; 77: 125-133.
12. Raini, M. dan Isnawati, A. *Kajian Khasiat dan Keamanan Stevia Sebagai Pemanis Pengganti Gula*, Media Litbang Kesehatan, 2011; 21(4): 145-156.
13. Shirwaikar, A., Parmar, V., Bhagat, J. and Khan, S. *Identification and estimation of stevioside in the commercial samples of stevia leaf and powder by HPTLC and HPLC*, International journal of pharmacy & life sciences, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, (Karnataka) – India, 2011; 2(9): 1050-1058.
14. Sigma-Aldrich, *Safety Data Sheet*, sigma-aldrich.com, 2014.
15. Supriyadi, Siswandono, Yuwono, M., Method Development and Validation for the Simultaneous Determination of stevioside, rebaudioside-A, rebaudioside-C and dulcoside-A contained in stevia rebaudiana bertoni using HPLC-ELSD, *International journal of pharmacy & life sciences*, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, (Karnataka) – India, 2016; 8(9): 1-5.