Impact Factor:					
ISRA (India)	= 4.971	SIS (USA)	= 0.912	ICV (Poland)	= 6.630
ISI (Dubai, UAE)	= 0.829	РИНЦ (Russia)	= 0.126	PIF (India)	= 1.940
GIF (Australia)	= 0.564	ESJI (KZ)	= 8.716	IBI (India)	= 4.260
JIF	= 1.500	SJIF (Morocco)	= 5.667	OAJI (USA)	= 0.350

SOI: 1.1/TAS DOI: 10.15863/TAS

International Scientific Journal

Theoretical & Applied Science

p-ISSN: 2308-4944 (print) **e-ISSN:** 2409-0085 (online)

Year: 2019 **Issue:** 12 **Volume:** 80

Published: 19.12.2019 **http://T-Science.org**

QR – Issue QR – Article

Oleg Ivanovych Yurchenko
Kharkiv V.N. Karazin National University
PhD, Full Professor of Chemical Metrology Department
yurchenko@karazin.ua

Tetyana Vasylivna Chernozhuk
Kharkiv V.N. Karazin National University
PhD, Associate Professor of Inorganic Chemistry Department
tanya.chernozhuk@gmail.com

Oleksii Andriovych Kravchenko
Kharkiv V.N. Karazin National University
PhD, Associate Professor of Chemical Metrology Department
alekseykravch@ukr.net

CHROMATOGRAPHIC DETERMINATION OF STEVIA IN SUGAR SUBSTITUTES WITH MEDICAL PROPERTIES

Abstract: *The method for controlling the quantitative content of stevioside using high-performance liquid chromatography in the processing and separation of stevioside from plant materials has been improved. This technique allows taking the mass analysis during the selection of plant samples for the content of other sweet glycosides. The principles of the process of purification of the obtained extract from plant stevia raw materials are determined based on desalination, decolorization of the extract without the use of expensive and toxic reagents. The influence on the degree of extraction of the following parameters, such as the degree of grinding of the raw materials, the ratio of raw materials-extractant, the temperature of the extractant, the number of sequential extracts, and their duration were studied for the optimization of the extraction process of stevioside. The greatest extraction of stevioside from plant materials is achieved by triple extraction of 20 ml., by boiling water for 30 minutes. The optimum particle size of the raw materials is 0.5 mm. Significantly, the receptors for the use of stevia as a source of low-calorie sweetening components in the production of dairy products for dietary use make it possible to obtain medically valuable cocktails, yogurts, and other products with high palatability.*

Key words: *stevia, thin layer chromatography, high-performance liquid chromatography, stevioside.*

Language: *English*

Citation: Yurchenko, O. I., Chernozhuk, T. V., & Kravchenko, O. A. (2019). Chromatographic determination of stevia in sugar substitutes with medical properties. *ISJ Theoretical & Applied Science, 12 (80),* 289-292.

Soi: http://s-o-i.org/1.1/TAS-12-80-59 ***Doi***: crossref https://dx.doi.org/10.15863/TAS.2019.12.80.59

Scopus ASCC: *1600.*

Introduction

Currently, one of the pressing health problems is the prevention and treatment of such widespread and intractable diseases as diabetes and hypoglycemia. In this regard, special attention is paid to the search for new low-calorie, effective and harmless sugar substitutes. Among the most promising and effective modern natural sweeteners, attention is drawn to sweet diterpenic glycosides that accumulate in the aerial part of the plant (from now on referred as stevia) in a rather large amount - 20% in terms of dry weight and having a pronounced sweet taste, which is in 250 to 300 times higher than the sweetness of sucrose.

Stevia is a perennial herbaceous plant belonging to the genus Stevia (sprout) of the Compositae family, consisting of a single or branched stalk 60-80 cm tall with cross-shaped elliptical leaves and small white flowers

collected in a lush inflorescence. Stevia grows in subtropical climatic conditions with an average daily temperature of 23 ° C on sandy soils. Dry stevia extracts, appearing on the world market under the names "Steviosin," "Stevix," are used either individually or in compositions with other agents for sweetening drinks and other food products. In many countries of the world - primarily in Japan, as well as Brazil, Korea, the USA, Paraguay, Laos, China, Indonesia, Thailand, and others - stevia extract sweeteners are used in a wide range. It is used in a variety of food products: wines, soft drinks, fruit berry syrups, confectionery, in the production of toothpaste, chewing gums, and cosmetic products.

Stevioside is the main diterpenic glycoside of stevia, non-toxic, low-calorie, and stable during heat treatment (up to 120 ° C). Provided that private technology for the production of alcoholic and non-alcoholic drinks, dairy products, bakery, and confectionery products, as well as mayonnaise sauces and preservatives, is used. Stevioside is the best suited for use in mixing processes in slightly acidic environments, in high-temperature processing processes and can be added to the recipe at any stage. The growing interest in stevia processed products and the appearance of stevioside-based food products and food additives on the market necessitate the development of effective methods for controlling their quality using modern analysis methods. Due to the extraordinary organoleptic properties and biological activity of stevia diterpenic glycosides, an in-depth study of the physicochemical and biological properties of these compounds is required, as well as improvement of methods for their isolation from plant materials.

In light of the preceding, the development of a simple, reliable, and universal method for controlling the content of stevioside in plant raw materials is of particular importance. Numerous methods have been published for determining stevioside, including capillary electrophoresis, gas-liquid chromatography, thin-layer chromatography with densitometry, gas chromatography, IR spectrometry. However, due to non-specificity, these methods are not widely used. A more attractive way of controlling the content of stevioside is to use HPLC due to the specificity of the determination of individual diterpene glycosides, as well as its simplicity and reliability. [1,p.122;2,p.43;3,p.88;4, p.100;5,p.74;6,p.43;7,p.20; 8, p.12;9,p.364;10,p.333;]

The purpose of the work is to develop a method for quantitative determination in plant materials, dry purified stevia extract, and food products using TLC and HPLC.

Preparation of raw materials and quality control Isolation of stevioside from plant materials

Isolation of dry purified stevia extract was made from dried stevia leaves purchased from Status (Kharkiv, Ukraine).

The dried chopped stevia leaves were extracted with 80% ethanol. The alcohol extract was evaporated to dryness, dissolved in water, and degreased with ethyl acetate. The sum of the diterpene glycosides was recovered from butanol from a defatted aqueous extract and recrystallized from methanol. In total, five laboratory series of the dry purified extract was obtained in a total amount of 10.9 g from 250 g of plant material (Table 1.).

Using HPLC,it was found that the content of stevioside in the dry purified extract of stevia is 66 - 72%. It was established that there are compounds belonging to the class of diterpenic glycosides, presumably to rebaudioside A and C, which are characterized by stronger retention on this sorbent.

Monitoring the content of stevioside in plant materials by HPLC and TLC

As an external standard, a sample of steviosidewas used, which we obtained in laboratory conditions from the leaves of Steviarebaudiana. TLC, HPLC confirmed the purity and authenticity of the sample. As prototypes used: dried leaves of Steviarebaudiana. TLC was carried out on FertigplattenKieselgel 60 (Merck) plates. HPLC was performed on a Stayer chromatographic system (NPLF Akvilon CJSC, Russia), consisting of two Marathon Series II pumps, a dynamic mixer, a Rheodyne 7725i injector with a loop volume of 10 µl and a UV-104 spectrophotometric detector with a fixed detection wavelength. Chromatographic information was controlled, collected, and processed using the Multichrom 2.0 software and hardware complex (Ampersand Russia). The separation was performed on a Luna NH_2 column (250 x 4.6 mm, 5 µm) (Phenomenex, United States). The Security Guard Cartidge NH_2 (4 x 3 mm) pre-column (Phenomenex, USA) was installed in the line in front of the analytical column in the universal holder of pre-columns. To ensure a constant temperature of the separation column, the Therma Sphere column thermostat (Phenomene, USA) was used. At the sample preparation stage, we used a 12-position vacuum manifold and Strata NH_2 solid-phase extraction cartridges (500 mg / 3 ml) (Phenomenex, USA), as well as anesthetized paper filters.

TLC method

1.0 g of ground (1 mm) raw material is placed in a 50 ml flask, 20 ml of 95% ethanol are added and heated in a boiling water bath under reflux for 15 minutes. The solution is filtered through a paper filter. The resulting solution is used for testing. 0.01 ml of the test solution and 1% solution of a standard sample of stevioside in methanol are applied to the starting line of the TLC plate with a micropipette or microsyringe, dried in air for 10 min and chromatographed in an ascending manner in a chamber with a solvent system: chloroform-methanol-water (in proportion of 60: 30: 6). After the front of the solvents has passed ~ 15 cm, the plate is removed from the chamber

and dried in air for 30 minutes, then the plate is sprayed with a 50% sulfuric acid solution. After keeping the plate in an oven at a temperature of 100 ° C for 15 minutes, black spots with different R_f values were identified on the chromatogram. Stevioside corresponded to a spot with Rf = 0.37, present both in the standard and in the test sample.

When developing the TLC technique, four types of systems were used: chloroform - methanol - water (60: 30: 6), chloroform - methanol - water (30: 10: 1), chloroform - methanol - water (30: 20: 1), butano - acetic acid-water (4: 1: 1). Various reagents were used for detection: 2% solution of vanillin in methanol - phosphoric acid (1: 1), 50% solution of sulfuric acid, anisic aldehyde - sulfuric acid - glacial acetic acid (0.5: 1: 50). The best results were obtained using the system of chloroform - methanol-water (60: 30: 6) and a 50% solution of sulfuric acid as a developer. Spots on the chromatograms of the test samples were evaluated in comparison with a standard sample of stevioside. The sensitivity of the determination procedure is 0.5 μg of stevioside.

IHPLC method

Separation is carried out at a column thermostat temperature of 50 ℃. Mobile phase: deionized water (pH 5.5) (A) - acetonitrile (B). Gradient program: 87-81.5% B in 12 min, 82.5 - 79% B in 8 min, 5% B - 5 min, 5% B - 5 min, 5% B - 87% B in 1 min, 87% B - 3 min. The volumetric rate is 1.5 ml / min. The detection wavelength is 210 nm.

Steviosideis extracted from dried and ground (0.5 mm) samples of plant materials (1 g) by extraction with triple water at a water temperature of 100 ° C. The volume of water at each stage of the extraction is 20 ml, and the extraction time is 30 minutes. For extraction, deionized water with a specific conductivity of not more than 0.2 μS C cm is used. The extracts are combined, cooled to room temperature, filtered through a paper filter, and their total volume is adjusted to 100 ml with deionized water. A 2 ml portion of the extract is passed through a solid-phase extraction cartridge, preconditioned with 3 ml of acetonitrile and 3 ml of deionized water. The first milliliter of the extract is discarded, and the subsequent portion of the purified extract is collected and used for analysis.

Organic solvents, such as methanol, ethanol, diethyl ether, and acetonitrile, were most often used as extractants for the isolation of diterpene glycosides. However, the use of these solvents is not only economically disadvantageous but also impractical from a chemical point of view, since a large amount of organic substances, primarily pigments that impede further analysis, passes into the extract. Extraction with water allows minimizing the content of related impurities. To optimize the extraction of stevioside, we studied the influence on the degree of extraction of such parameters as the degree of grinding of the raw materials, the ratio of raw materials to extractant, extractant temperature, the number of sequential extracts, and their positivity. The most significant extraction from plant materials provides a triple extraction of 20 ml of boiling water for 30 minutes. The optimum particle size of the raw materials is 0.5 mm.

Quality control and standardization

The final products manufactured according to these specifications must have a conclusion of the state sanitary and epidemiological examination and in terms of safety for human health must meet the following requirements:

- microbiological indicators: MAFAM (KUSCH in 1g, not more): 1000; BGKP (coliforms) 1g - not allowed; pathogenic microorganisms, including bacteria of the genus Salmonella in 25g - not allowed; yeast, KUO in 1g - not more than 50 (in tablets - not more than 10) according to 3 clause 3.4 of TU U 15.6-30883300-009: 2007 “Natural sweeteners. Technical conditions.”;

- allowable levels of the composition of toxic elements, micro toxins, in mg / kg, not more: lead-1.0; cadmium-0.05; arsenic-0.5; mercury-0.01; copper 4.0; Zinc-8.0 according to clause 3.5 of TU 15.6-30883300-009: 2007 Natural sweeteners. Technical conditions.”;

- permissible levels of pesticide composition are standardized following the requirements of DSanPiN№8.8.1.2.3.4.-000-2001 “Permissible doses, concentrations, quantity and composition levels of pesticides in agricultural raw materials, food products”;

- permissible levels of radionuclide composition, in BG/kg, not more than 137 Cs-150 and 90Sr-50, according to GN 6.6.1.1-130-2006 “Permissible levels of radionuclide composition 137 Cs-150 and 90Sr in food and drinking water”.

Thus, methods have been developed to control the quantitative content of stevioside by high-performance liquid and thin-layer chromatography in the process of technological processing and the separation of stevioside from plant materials. They make it possible to carry out mass analyzes for the content of other sweet glycosides.

Table 1. The results of obtaining of the dry purified extract from stevias leaves

Number	Weight, g	An analysis of steviozyd	Weight of the dry extract	An analysis of steviozyd in the dry extract
1	50	4,04	2,5	68,2
2	50	4,04	2,1	67,3
3	50	4,04	1,5	71,3
4	50	4,04	2,9	
5	50	4,04	1,9	
Total	250		10,9	

References:

1. Mitchell, H. (2002). *Podslastiteli*. (p.211). SbB, Professiya.
2. Kedic, S.A. (2002). *Pitanie i zdorovie. V.8(1)*, pp. 42-44.
3. Polenskii, K. (2004). *Food industry. V9*, pp.87-88.
4. Kedic, S.A. (2003). *Pitanie i zdorovie. V.3 (6)*, pp. 97-101.
5. Sinnichuk, I., & Strizeva, Ye. (2002). *Himiya rastitelnogo syria. V.3 (6)*, pp. 73-75.
6. Holtoev, F.T. (2003). *Him-farm. journal. V.37 (6)*, pp. 42-45.
7. Kedic, S.A. (2003). *Him-farm. journal V.37 (10)*, pp. 19-22.
8. Podporinova, G.K. (2006). *Podslastiteli i saharozameniteli.* (p.156). Voronez, FGOUProfessiya.
9. Cindrić, I.J., et al. (2011). *Microchemical Journal, V.1(99)*, pp. 364 – 369.
10. Pereira Junior, J.B., & Dantas, K.G.F. (2016). *Food Chemistry, V. 1(196)*, pp.331 – 337.