IJPSR (2025), Volume 16, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 April 2025; received in revised form, 08 May 2025; accepted, 14 June 2025; published 01 October 2025

STABILITY OF REBAUDIOSIDE M UNDER ACIDIC CONDITIONS AND ITS DEGRADATION PRODUCTS

Indra Prakash *, Juvenal Higiro, Laura Martin and Lauren Seabrook

Flavor and Ingredient Research, The Coca-Cola Company, One Coca-Cola Plaza, Atlanta, GA 30313, Atlanta, Georgia, USA.

Keywords:

Stevia, Reb M, Stability

Correspondence to Author:
Indra Prakash

Research Fellow-Sweetener,
Flavor and Ingredient Research,
The Coca-Cola Company,
One Coca-Cola Plaza, Atlanta, GA 30313, Atlanta, Georgia, USA.

E-mail: iprakash@coca-cola.com

ABSTRACT: Current study aims to evaluate stability of Rebaudioside M, a sweet component of *Stevia rebaudiana*, using a typical pH (2.8, 3.2, 3.8 and 4.2) and temperature (5 °C, 20 °C, 30°C, 40 °C) for 26 weeks, range that simulated both relevant and extreme beverage storage conditions. Photostability of mock beverages at pH 3.8 was determined at 25 °C /60% relative humidity (RH) with fluorescent light exposure. Rebaudioside M (Reb M) was evaluated in mock beverage solutions by simulating formulations used in commercial soft drinks and were subjected to various temperature conditions (5 °C, 20 °C, 30 °C, 40 °C) for 26-weeks at each pH. Samples were analyzed at scheduled intervals throughout the 26 weeks for Reb M, known impurities, known degradation products, and unidentified compounds greater than or equal to 0.100% of the starting concentration of Reb M. There was minimal degradation of the Reb M when exposed to fluorescent light, and appearance did not change throughout the study. Thus, Reb M in mock beverages under relevant conditions of intended use is considered stable. Finally, Reb M sourced from 1) extraction of Stevia leaf, 2) bio-conversion of Reb A and 3) fermentation of glucose were evaluated by a trained panel for taste, aftertaste, and mouth feel qualities. Samples were evaluated at the levels of 100ppm and 300ppm, both at 4°C and at ambient temperature. Sensory evaluation revealed no significant differences in sensory profile by source, suggesting that the samples tasted similarly to each other.

INTRODUCTION: *Stevia rebaudiana* (Bertoni), is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil) [1, 2]. Several sweet compounds such as Rebaudiosides A, B, C, D, E, M, N, O, I, Stevioside, and Dulcoside A; have been reported in the Stevia plant. These compounds are all glycosides of the diterpene ent-13-hydroxykaur-16-en-19-oic acid and are known as steviolglcyosides [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.16(10).2739-52

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(10).2739-52

The leaves of *Stevia rebaudiana* Bertoni have been used by the natives of Paraguay to sweeten beverages for centuries [5]. Rebaudioside M (Reb M) (6) is a steviol glycoside that is considered to have fewer negative perceptual features (e.g., long linger, bitterness) and tastes about 200–350x sweeter than sucrose. It is a glycoside of the ent-kaurene diterpenoid aglycone known as steviol and is found in nature accompanied by at least ten other sweet-tasting steviol glycosides [7-9].

We have isolated several steviolglycosides from commercial extracts of the leaves of *Stevia rebaudiana* obtained from various suppliers around the world [10-16]. Stevia sweeteners have been approved for use as a sweetener in several countries, including US, EU, Japan, China, Brazil and other countries.

The structures of Rebaudiosides were not fully determined until 1970 [17, 18]. During the 1970s, additional sweet components, including Rebaudiosides A–E, were isolated from *Stevia rebaudiana* leaves and characterized by Osamu Tanaka and co-workers at Hiroshima University in Japan [19]. Several novel steviol glycosides have been reported from the commercial extracts of the leaves of *Stevia rebaudiana* in the last few years [6, 11-16, 20-23]. Recently we reported the structure elucidation and isolation of Reb M from *Stevia rebaudiana* [6, 24, 25]. In addition, Reb M received a Letter of No Objection concerning its Generally Recognized as Safe (GRAS) status from US FDA (GRAS Notice, 2013).

We are interested in sweetness, sweetness enhancement, stability and physicochemical properties of steviol glycosides in various systems of interest, and the identification of degradation products using various spectroscopic analysis [10, 26]. The primary objective of this study was to assess the stability of Reb M covering a typical pH range of 2.8 to 4.2 at various temperatures that simulated both relevant and extreme beverage storage conditions. Literature search indicated several reports on the stability of steviol glycosides under various conditions [27-30].

An additional goal was to evaluate the overall taste quality of Reb M generated from different sources (extraction of Stevia leaf, bio-conversion of Reb A and fermentation of glucose) to determine if there were source-specific differences in taste. Reb M at 100 and 300ppm were evaluated by a trained panel for taste, aftertaste, and mouth feel qualities, both at 4°C and at ambient temperature.

In the present study, stability of Rebaudioside M was evaluated in carbonated mock beverage solutions by simulating formulations used in commercial cola soft drinks (pH 2.8 and pH 3.2), lemon–lime soft drinks (pH 3.8), and root beer soft drinks (pH 4.2) but lacking flavour components. Also, the mass (mole) balances of the mixture of steviol glycosides and its major degradation products obtained during the course of study are reported. Also, this marks the first report that Reb M obtained from either *via* extraction of Stevia leaf [31], bio-conversion of Reb A [32] or by fermentation of glucose [2] have the same sensory profile.

MATERIALS AND METHODS:

HPLC MS/MS: HPLC MS/MS detection was used to detect impurities in Rebaudioside M (6). Samples were prepared in acetonitrile buffer solution and diluted if appropriate. The analytes were chromatographed using reversed phase high performance liquid chromatography with tandem mass spectrometry (HPLC MS/MS) detection. The LOQ for all analytes is 0.01% of the Rebaudioside M theoretical concentration in an approximately 500mg/L solution.

Solutions: Glacial acetic acid (AcOH) was obtained from EMD (Gibbstown, NJ), ammonium acetate (NH4OAc) was from Fluka (a part of Sigma- Aldrich, Bellefonte, PA), and 85% phosphoric acid (H3PO4) was from Fisher Scientific (Pittsburgh, PA), all of which were reagent grade. HPLC grade acetonitrile (MeCN) was purchased from Burdick & Jackson (Muskegon, MI). Water was purified using a Millipore system (Billerica, MA).

Mobile Phase: All solvents were degassed for at least two minutes with helium before use. The HPLC method employed was a two solvent mobile phase system: Solvent A 0.1% phosphoric acid was prepared by dissolving 1g of concentrated phosphoric acid to 1000ml with ultra-pure water or Solvent A acetate buffer for CAD (0.0284% NH4OAc, 0.0116% AcOH) which was prepared by dissolving 0.569 g of NH4OAc and 0.231 ml of AcOH in two liters of purified water and mixing thoroughly (pH: 5.0); Solvent B was 100% MeCN; was prepared by adding 0.4 ml of AcOH to one liter of purified water by mixing thoroughly.

Method: Rebaudioside M and its associated impurities and degradants were determined in beverage using UV detection or charged aerosol detection (CAD). An Agilent (Wilmington, DE) 1200 HPLC, including a gradient pump, a temperature-controlled column compartment capable of maintaining 55°C, an auto sampler and a UV absorbance detector, was used for the analysis. A Charged Aerosol Detector (CAD), ESA, Inc. (Chelmsford, MA), was also used for the analysis. The scale on the CAD was 100 pA and the filter was set to medium. The switching valve diverted the first 5.5 min of each injection away from the CAD detector to prevent fouling of the detector.

The system was controlled using Waters (Milford, MA) Empower software. The RP-HPLC employed a Phenomenex (Torrance, CA) Synergi-Hydro column (250 mm× 4.6 mm, 4 µm) with a Phenomenex Security guard C18 cartridge and a tertiary solvent mobile phase as shown in **Table 3.** The column was at a temperature of 55 °C and the flow rate was 1.5 ml/min. The injection volume of each sample was 24 µl, or 15 µl (75 µl, for beverage impurity analysis) which were kept at ambient temperature while in the auto sampler. In all cases for UV detection, a 16 nm bandwidth was used with a reference wavelength of 650 nm (100 nm band width). CAD was used for the analysis of all steviol glycosides with a total run time of 43 min. For the RP-HPLC method, the column was flushed with 50 ml of 90% MeCN to waste before use and the samples were bracketed with standards by injecting them at the beginning and at the end of a run for accuracy of their retention times. The details of the solvents used for the RP-HPLC gradient method for the identification of Rebaudioside M, its known impurities and degradation products was given in **Table 1.** Each analyte was identified by retention time matching with reference standards. The concentration of each analyte was also calculated using the method described earlier [27]. The initial concentrations of the five compounds 1, 5, 6, 7, 9 at time zero are given in **Table 3.**

Samples are prepared in acetonitrile buffer solution and diluted if appropriate. Beverage may be injected with no additional preparation after degassing as needed. The analytes are chromatographed using reverse phase chromatography with detection at 210nm and or CAD. Analytes are separated using a gradient consisting of 0.1% phosphoric acid versus 100% acetonitrile. Rebaudioside M is quantitated against a Rebaudioside M standard. Stevioside and other impurities or degradants are quantified against a stevioside standard. As reference standards for some know impurities are not available, they are identified by retention time and quantitated using molecular weight correction factor. If CAD is used an acetate buffer mobile phase is substituted for the 0.1% phosphoric acid mobile phase.

TABLE 1: RP-HPLC METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF STEVIOL GLYCOSIDES

Time (min)	% A	% B	Flow (ml/min)	C
0	75	25	1.5	Initial
34	61	39	1.5	Linear
34.1	10	90	1.5	Linear
38	10	90	1.5	Hold
38.1	75	25	1.5	Linear
43	75	25	1.5	Hold

Structure and Reference Standards and Materials: The Rebaudioside M structure is shown in **Fig. 1.** Rebaudioside M (6) used in this study is a mixture containing mainly Rebaudioside M (6) along with minor quantities of other compounds namely 1, 5, and 9 except at pH 3.2 which contains 7. The compounds present in the Rebaudioside M mixture and degradation productsin this study are as shown in **Fig. 2** & **Fig. 3.**

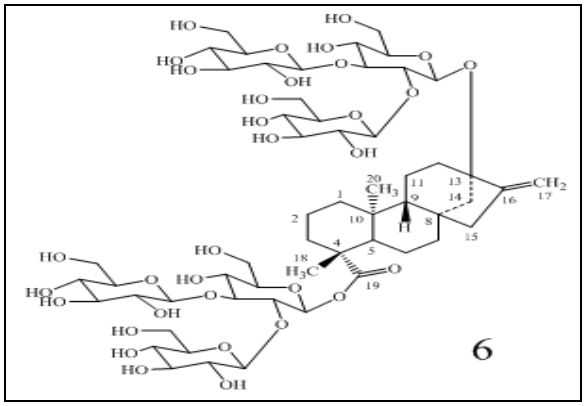

FIG. 1: REBAUDIOSIDE M STRUCTURE

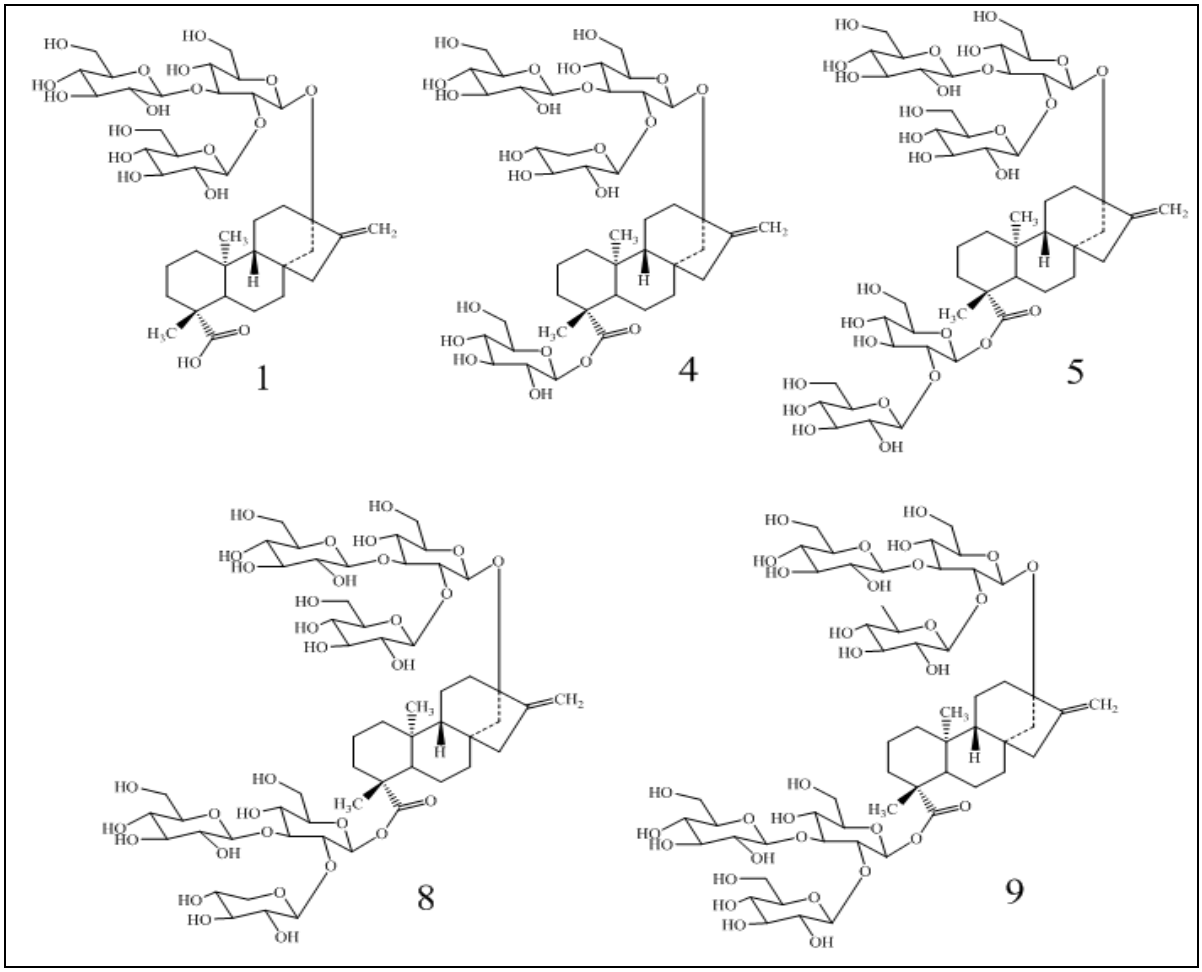

FIG. 2: STRUCTURE OF STEVIOL GLYCOSIDEWITH STEVIOL

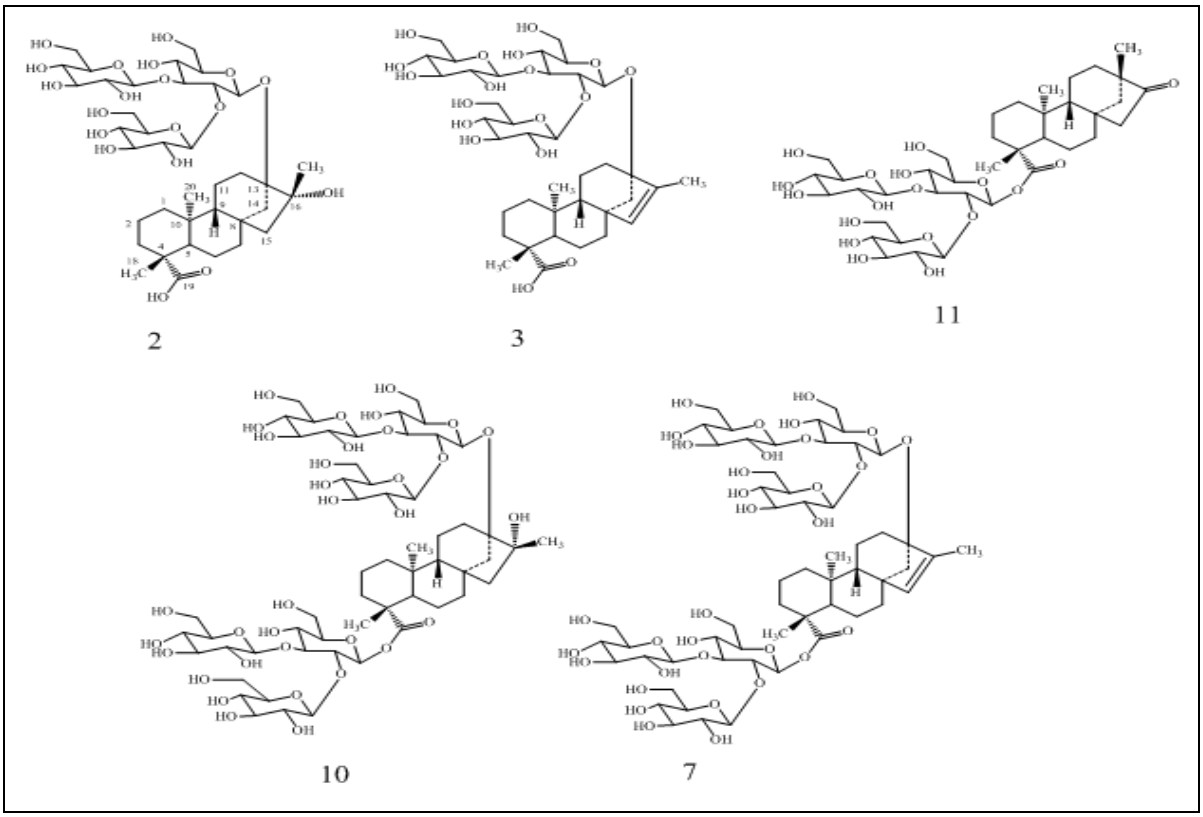

FIG. 3: STRUCTURE OF STEVIOL GLYCOSIDE WITH NON-STEVIOL

Moisture Analysis by Karl Fischer: For Karl Fischer moisture analysis, titration was performed using a Met Rohm 784 KFP Titrino titrator. The pH of one liter of water was adjusted to 3.3 with glacial AcOH to make diluent buffer. The diluent solution was prepared by mixing 250 ml of MeCN with 750 ml of the diluent buffer. It was then allowed to come to room temperature. The moisture content was measured by Karl Fischer titration each time the standards were prepared. This was necessary each time because of the hygroscopic nature and the fact that these compounds easily gain or lose moisture with changes in humidity. The standards were prepared by weighing 21.0, 30.0, 39.0, 48.0, and 60.0 (each± 0.5) mg in separate 100 ml volumetric flasks, diluting to volume with the diluent solution and stirring, if necessary, until dissolved. The concentrations were corrected for moisture and purity. The standard compounds were injected once at the beginning and once at the end of the sequence; usually the standards are stable for 2 months when stored in a refrigerator at 5 ± 3 °C.

Preparation of Mock Beverage Samples, Carbonation and Bottling: Bottles of mock beverages containing deionized water, potassium benzoate, tri-sodium citrate (dihydrate), potassium chloride, sodium chloride, magnesium chloride, and calcium sulfate (anhydrous) were prepared at pH 2.8, 3.2, 3.8 and 4.2 using phosphoric acid. All the reagents used are of reagent grade and were purchased from Fisher Scientific (Pittsburgh, PA). Eight different formulations representing 2 products (one with Rebaudioside M at a concentration of about 500 mg/l and the other one without Rebaudioside M) at each pH 2.8, 3.2, 3.8 and 4.2 were prepared. The formulations of mock beverages with and without Rebaudioside M are shown in **Table 2.**

TABLE 2: FORMULATION FOR BEVERAGE MATRIX

Without Rebaudioside M		With Rebaudioside M	
Ingredients	**Weight (g)**	**Ingredients**	**Weight (g)**
Deionized Water	49812.27	Deionized Water	49787.35
Potassium Benzoate	25.2	Potassium Benzoate	25.2
Trisodium Citrate (dehydrate)	11.475	Trisodium Citrate (dehydrate)	11.475
Potassium Chloride	0.765	Potassium Chloride	0.765
Sodium Chloride	0.953	Sodium Chloride	0.953
Magnesium Chloride	4.325	Magnesium Chloride	4.325
Calcium Sulfate	4.55	Calcium Sulfate	4.55
		Rebaudioside M	25

The products were prepared in a stainless-steel kettle with a propeller mixture. The deionized water, which was pre-chilled in a refrigerated room at 4–5 °C, was weighed and added first to the mixing kettle. The dry ingredients, phosphoric acid, which were pre-weighed into separated containers, were added sequentially into the mixing kettle to obtain mock beverage samples. Each product was placed into a Zahm & Nagel carbonator unit. The air was purged from the tank by sparging the product with CO_2 and bleeding off the headspace gas. The carbonator tank was then sealed, placed into an ice water bath and pressurized with CO_2. The product was carbonated by adjusting the temperature and CO_2 pressure to levels that corresponded to 3.8 + 0.2 volumes of carbonation. Carbonation level was tested using a Zahm DT piercing device. When the product met the desired carbonation range, the product was bottled, sealed with a crimpon crown closures, and then placed into refrigerated storage. All the products were stored refrigerated by Covance in a desiccator at 5±3 °C. The stability of Rebaudioside M was evaluated in mock beverage solutions lacking flavour components by simulating the above eight formulations at temperatures 5±3 °C, 20±2 °C, 30±2 °C, and 40±2 °C. Two sets of mock beverages at each pH under four temperatures were prepared and analyzed in duplicate using the HPLC method as stated above for Rebaudioside M, their known impurities and degradation products, as well as unidentified compounds that are greater than or equal to 0.100% from the starting concentration of Rebaudioside M at scheduled intervals (0, 1, 2, 6, 12, 18 and 26 weeks) throughout the 26 weeks. All samples were treated identically during analysis to minimize assay bias.

Fluorescent of Light Storage: At the 0-week testing interval, two Rebaudioside M (6) in mock

beverage bottles (pH 3.8) were covered with plastic wrap and two were covered with plastic wrap and aluminum foil. Bottles were placed on their side and exposed to a minimum, 1.2 million lux hours and not less than 200 watts hours/m^2exposure to near ultra-violet light at 25°C.

Sensory Evaluation of Rebaudioside M: A panel of trained tasters evaluated Rebaudioside M samples from three sources: 1) extracted from Stevia leaf, 2) bio-converted from Reb A, and 3) fermented from glucose. The samples were evaluated at two different concentrations (100 and 300ppm) and two different temperatures (4°C and ambient temperature). Evaluators were trained panelists (n = 14) with extensive experience tasting sweeteners and sweetener blends. Samples were profiled for sweetness, bitterness, sourness (taste qualities), drying (mouthfeel quality), and sweet, bitter, or sour aftertaste (taste quality – temporal). Briefly, panelists were asked to take a sip of the sample (~10mL), evaluate it for the attribute of interest, and make their evaluation on the perceived strength of the attribute of interest on a 15-point scale, where 0 is Not Present and 15 is Strongest Possible. Scores were analyzed for differences by ANOVA with follow up post-hoc Tukey's HSD tests. For representation, rating means are used in the Results section.

RESULTS AND DISCUSSION: Table 3 shows 0-week Rebaudioside M results. The 0-week results for each pH level were considered acceptable since each mean Rebaudioside M concentration had a relative standard deviation (RSD) of <5%.

TABLE 3: 0-WEEK STABILITY DATA

			Compounds				
		Total Sum	**1**	**5**	**6**	**7**	**9**
Mock Beverage pH 2.8	Average	480	11.1	5.5	463	-	1.12
	SD	1.4	0.44	0.107	1.2	NA	0.067
	RSD%	0.3	3.9	2	0.3	NA	6
Mock Beverage pH 3.2	Average	483	11.5	5.56	464	0.587	1.34
	SD	1.1	0.27	0.185	0.9	0.0664	0.197
	RSD%	0.2	2.3	3.3	0.2	11.3	14.7
Mock Beverage pH 3.8	Average	482	11.2	5.49	465	-	1.05
	SD	0.7	0.22	0.093	0.8	NA	0.165
	RSD%	0.1	2	1.7	0.2	NA	15.8
Mock Beverage pH 4.2	Average	479	11.1	5.53	461	-	1.33
	SD	3.2	0.15	0.105	3.2	NA	0.213
	RSD%	0.7	1.4	1.9	0.7	NA	16.1

From the HPLC analysis data **Table 4A-4D,** it was indicated that the stability of Rebaudioside M in mock beverage solutions was pH, temperature, and time dependent.

TABLE 4A: CONCENTRATION OF EACH ANALYTE IN MOCK BEVERAGE AT PH 2.8 (mg/L) UNDER VARIOUS TEMPERATURE. RESULTS ARE MEAN OF THREE SAMPLE PREPARATIONS

			Compounds										
Temperature	**Week**	**% 0-Week (6)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	0	N/A	11.1	–	–	–	5.5	463	–	–	1.12	–	–
5 °C	1	100.2	11.2	–	–	–	5.9	464	0.896	0.633	1.59	–	–
pH 2.8	2	99.1	11	–	–	–	5.61	459	0.599	–	1.29	–	–
	4	100.6	11.7	–	–	–	5.67	466	1.12	0.568	1.64	–	–
	6	100.2	12.5	–	–	–	5.88	464	1.4	–	1.64	–	–
	8	100.4	11.4	–	–	–	5.76	465	1.29	–	1.14	0.501	–
	10	100.2	10.5	–	–	–	5.4	464	1.46	–	1.07	0.564	–
	12	99.8	10.7	–	–	–	5.24	462	1.58	–	1.2	–	–
	16	99.8	10.5	–	–	–	5.4	462	2.05	–	1.14	0.639	–
	18	99.8	10.7	–	–	–	5.68	462	2.09	–	1.28	0.698	–
	26	99.4	11	–	–	–	5.69	460	2.85	–	1.27	0.746	–
20 °C	0	N/A	11.1	–	–	–	5.5	463	–	–	1.12	–	–
pH 2.8	1	100	12.5	–	–	–	6.09	463	1.94	0.529	1.3	0.739	–
	2	98.9	11.2	–	–	–	5.55	458	2.38	–	1.33	0.698	–

	4	99.4	11.2	–	–	–	5.6	460	4.13	–	1.31	1.27	–
	6	97.8	12.9	–	–	–	5.67	453	6.33	–	1.21	1.81	–
	8	97.8	11.9	–	–	–	5.67	453	7.95	–	1.07	2.21	–
	10	97.4	11.4	–	–	–	5.22	451	9.46	–	1.1	2.5	–
	12	96.3	12.1	–	–	–	5.16	446	11	–	1.09	2.94	–
	16	95.7	12.9	–	–	–	5.3	443	14.9	–	1.13	3.97	–
	18	95.2	13.1	–	–	–	5.46	441	16.8	–	1.19	4.6	–
	26	92.9	13.9	–	0.737	–	5.29	430	23.1	–	1.23	6.46	–
30 °C	0	N/A	11.1	–	–	–	5.5	463	–	–	1.12	–	–
pH 2.8	1	98.9	11.6	–	–	–	5.87	458	4.3	0.658	1.64	1.48	–
	2	97	11.8	–	–	–	5.47	449	7.6	–	1.17	2.23	–
	4	95.2	13.6	–	0.719	–	5.41	441	15.6	–	1.6	4.75	–
	6	92.9	16.1	–	1.29	–	5.59	430	22.3	–	0.731	6.46	–
	8	90.7	15.5	–	0.995	–	5.23	420	29.1	–	1.03	8.55	–
	10	89.8	15.5	–	1.06	–	4.64	416	32.8	–	0.988	9.38	0.522
	12	86	15.7	–	1.66	–	4.1	398	35.2	–	0.936	9.61	0.53
	16	82.3	19.3	0.531	2.53	–	4.46	381	51.8	–	0.988	14.5	0.734
	18	80.3	20	0.714	3.04	–	4.45	372	59.9	–	1.1	17	0.981
	26	72.8	22.2	1.4	4.62	–	3.96	337	76.4	–	1.07	21.9	1.12
40 °C	0	N/A	11.1	–	–	–	5.5	463	–	–	1.12	–	–
pH 2.8	1	96.1	13.9	–	–	–	6.04	445	11.7	0.67	1.44	3.57	–
	2	92	15	–	0.797	–	5.11	426	21.8	–	1.29	6.09	–
	4	85.1	18.2	–	2.12	–	4.93	394	41.2	0.502	1.08	12.4	1.15
	6	78.4	23.9	1.03	3.94	–	4.44	363	54.8	–	1.06	17.1	1.09
	8	72.1	24.2	1.37	4.93	–	4.09	334	73.4	–	0.911	22.8	1.37
	10	66.5	24.6	1.65	6.22	0.548	3.37	308	81.1	–	0.835	25.1	1.66
	12	61.3	27.4	2.5	8.96	–	3.12	284	95.9	–	0.737	29	2.03
	16	52.5	29.6	4.11	13.8	–	2.73	243	114	–	0.671	36.1	2.63
	18	48.2	31.1	5.56	16.9	–	2.59	223	128	–	0.667	41.6	2.93
	26	34.1	31.6	9.6	26.9	–	1.99	158	145	–	0.554	54.2	3.85

TABLE 4B: CONCENTRATION OF EACH ANALYTE IN MOCK BEVERAGE AT PH 3.2 (mg/L) UNDER VARIOUS TEMPERATURE. RESULTS ARE MEAN OF THREE SAMPLE PREPARATIONS

			Compounds										
Temperature	**Week**	**% 0-Week (6)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	0	N/A	11.5	–	–	–	5.56	464	0.587	–	1.34	–	–
5 °C	1	99.8	10.9	–	–	–	6.04	463	0.904	0.568	1.4	–	–
pH 3.2	2	100.2	11.3	–	–	–	5.59	465	0.887	–	1.2	–	–
	4	100.9	10.3	–	–	–	5.63	468	0.89	–	1.44	–	–
	8	100.9	11.1	–	–	–	5.63	468	0.856	–	1.17	–	–
	10	100.6	10.1	–	–	–	5.22	467	0.947	–	1.04	–	–
	12	100	10.6	–	–	–	5.47	464	0.986	–	1.15	–	–
	16	101.1	10.5	–	–	–	5.59	469	1.28	–	1.18	–	–
	18	100.6	10.3	–	–	–	5.23	467	1.22	–	1.25	–	–
	26	100.2	10.3	–	–	–	5.41	465	1.49	–	1.15	0.514	–
20 °C	0	N/A	11.5	–	–	–	5.56	464	0.587	–	1.34	–	–
pH 3.2	1	99.6	10.8	–	–	–	6.17	462	1.27	–	1.81	–	–
	2	100.2	11.5	–	–	–	5.65	465	1.38	0.518	1.37	–	–
	4	100.4	11	–	–	–	5.55	466	2.3	–	1.37	0.666	–
	8	99.8	11.6	–	–	–	5.75	463	3.85	–	1.16	1.11	–
	10	99.6	10.6	–	–	–	5.18	462	4.66	–	1.08	1.33	–
	12	98.5	11.2	–	–	–	5.2	457	5.31	–	1.2	1.47	–
	16	98.9	11.5	–	–	–	5.38	459	7.07	–	1.1	2	–
	18	98.5	11.7	–	–	–	5.26	457	7.5	–	1.22	2.12	–
	26	96.8	11.9	–	–	–	5.34	449	11	–	1.05	3.06	–
30 °C	0	N/A	11.5	–	–	–	5.56	464	0.587	–	1.34	–	–
pH 3.2	1	98.9	10.6	–	–	–	5.92	459	2.34	0.702	1.59	1.11	–
	2	99.1	11.8	–	–	–	5.26	460	3.79	–	1.15	1.09	–
	4	98.5	12..6	–	–	–	5.37	457	7.11	0.61	1.52	2.32	–
	8	96.1	13.4	–	–	–	5.4	446	13.8	–	1.12	3.89	–

	10	95.5	12.9	–	–	–	4.98	443	16.8	–	1.07	4.6	–
	12	93.5	13.8	–	0.594	–	4.41	434	19.9	–	1.09	4.77	–
	16	91.8	15.1	–	1.02	–	4.86	426	25.9	–	1.07	7.19	–
	18	90.1	16.1	–	1.3	–	4.52	418	28.2	–	1.06	7.99	–
	26	84.9	16.7	–	1.86	–	4.64	394	41	–	1.09	12.3	0.522
40 °C	0	N/A	11.5	–	–	–	5.56	464	0.587	–	1.34	–	–
pH 3.2	1	98.1	12.6	–	–	–	5.71	455	6.28	0.696	1.85	2.11	–
	2	96.1	13.3	–	–	–	5.46	446	11.2	–	1.26	3.33	–
	4	92.9	15.2	–	1.03	–	5.12	431	22.1	–	1.88	6.7	0.782
	8	85.3	20.2	0.526	1.97	–	4.34	396	39.8	–	0.995	10.9	0.699
	10	81.9	20.2	0.565	2.19	–	4.19	380	46.9	–	0.937	14.1	0.865
	12	77.6	22.9	0.933	3.5	–	3.99	360	57.3	–	0.979	17.3	1.11
	16	72.2	25.5	1.61	5.29	–	3.78	335	72.5	–	0.932	21.4	1.37
	18	67.7	27.2	1.9	6.65	–	3.44	314	78.5	–	0.901	23.3	1.67
	26	57.1	31.9	3.82	11.9	–	3.22	265	3-Jan	–	1.06	32.6	2.54

TABLE 4C: CONCENTRATION OF EACH ANALYTE IN MOCK BEVERAGE AT PH 3.8 (mg/L) UNDER VARIOUS TEMPERATURE. RESULTS ARE MEAN OF THREE SAMPLE PREPARATIONS

			Compounds										
Temperature	**Week**	**0-Week (6)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	0	N/A	11.2	–	–	–	5.49	465	–	–	1.05	–	–
5 °C	1	99.8	13	–	–	–	6.21	464	0.56	0.681	1.77	–	–
pH 3.8	2	99.6	11.1	–	–	–	5.58	463	–	–	1.22	–	–
	4	100.2	11.7	–	–	–	5.91	466	–	–	1.61	–	–
	8	100.6	11	–	–	–	5.93	468	–	–	1.26	–	–
	10	99.6	9.62	–	–	–	5.33	463	0.503	–	1.09	–	–
	12	100	10.6	–	–	–	5.41	465	–	–	1.16	–	–
	16	99.8	10.4	–	–	–	5.57	464	0.54	–	1.15	–	–
	18	100.2	10.5	–	–	–	5.45	466	–	–	1.25	–	–
	26	99.6	10.9	–	–	–	5.93	463	0.615	–	1.23	–	–
20 °C	0	N/A	11.2	–	–	–	5.49	465	–	–	1.05	–	–
pH 3.8	1	99.6	12.3	–	–	–	6.49	463	0.677	0.817	1.86	–	–
	2	99.4	10.7	–	–	–	5.83	462	0.518	–	1.51	–	–
	4	99.6	10.2	–	–	–	5.64	463	0.835	0.588	1.5	–	–
	8	99.8	11.8	–	–	–	5.84	464	1.11	–	1.18	–	–
	10	100	9.96	–	–	–	5.41	465	1.36	–	1.12	–	–
	12	99.4	10.7	–	–	–	5.32	462	1.45	–	1.27	0.57	–
	16	99.6	11	–	–	–	5.41	463	1.82	–	1.21	0.569	–
	18	99.4	10.3	–	–	–	5.5	462	2.01	–	1.23	0.682	–
	26	99.1	11.7	–	–	–	5.72	461	2.93	–	1.28	0.794	–
30 °C	0	N/A	11.2	–	–	–	5.49	465	–	–	1.05	–	–
pH 3.8	1	99.8	11.5	–	–	–	6.48	464	1.18	0.726	1.66	–	–
	2	99.4	11.3	–	–	–	5.55	462	1.18	–	1.46	–	–
	4	99.4	11.9	–	–	–	5.54	462	2.17	0.613	1.5	–	–
	8	99.1	12	–	–	–	5.82	461	3.68	–	1.15	1.1	–
	10	98.7	11.4	–	–	–	5.33	459	4.34	–	1.04	1.22	–
	12	98.1	10.9	–	–	–	4.73	456	4.31	–	1.02	1.28	–
	16	98.1	12.7	–	–	–	5.41	4456	6.82	–	1.08	1.88	–
	18	97.6	12.6	–	–	–	5.4	454	6.8	–	1.24	1.97	–
	26	95.9	14.1	–	–	–	5.65	446	10.9	–	1.15	3.09	–
40 °C	0	N/A	11.2	–	–	–	5.49	465	–	–	1.05	–	–
pH 3.8	1	99.1	13.9	–	–	–	6.48	461	2.34	0.628	1.71	0.994	–
	2	98.5	11.8	–	–	–	5.47	458	3.47	–	1.27	1.01	–
	4	97.6	14	–	–	–	5.47	454	6.89	–	1.51	1.84	–
	8	95.3	15.9	–	–	–	5.45	443	13	–	1.08	3.29	–
	10	94	15.1	–	–	–	5.07	437	15.7	–	1.06	4.34	–
	12	92.7	17.2	–	0.631	–	4.97	431	18.1	–	1.1	4.95	–
	16	90.5	19.1	–	1.08	–	4.95	421	24.4	–	1.13	6.77	–
	18	89	20.1	–	1.32	–	4.75	414	26.9	–	1.27	7.57	–
	26	83.4	24.8	0.553	2.37	–	5.13	388	8-Feb	–	1.21	11.5	0.65

TABLE 4D: CONCENTRATION OF EACH ANALYTE IN MOCK BEVERAGE AT PH 4.2 (mg/L) UNDER VARIOUS TEMPERATURE. RESULTS ARE MEAN OF THREE SAMPLE PREPARATIONS

			Compounds										
Temperature	**Week**	**% 0-Week (6)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	0	N/A	11.1	–	–	–	5.53	461	–	–	1.33	–	–
5 °C	1	100	11.5	–	–	–	6.49	461	–	0.571	1.59	–	–
pH 4.2	2	100.7	10.3	–	–	–	5.47	464	–	–	1.47	–	–
	4	101.5	11.1	–	–	–	5.85	468	–	–	1.22	–	–
	8	101.5	11	–	–	–	4.98	468	–	–	1.09	–	–
	10	102	9.25	–	–	–	5.31	470	–	–	1.05	–	–
	12	100/2	9.97	–	–	–	5.31	462	–	–	1.15	–	–
	16	100.9	8.89	–	–	–	5.55	465	–	–	1.12	–	–
	18	101.1	10.6	–	–	–	5.36	466	–	–	1.27	–	–
	26	100.9	9.74	–	–	–	5.52	465	–	–	1.23	–	–
20 °C	0	N/A	11.1	–	–	–	5.53	461	–	–	1.33	–	–
pH 4.2	1	100.2	11.2	–	–	–	6.62	462	0.58	0.678	1.66	–	–
	2	100.2	11.4	–	–	–	5.56	462	–	–	1.14	–	–
	4	101.3	11	–	–	–	5.59	467	0.51	–	1.49	–	–
	8	101.5	11.3	–	–	–	5.81	468	0.661	–	1.12	–	–
	10	101.5	10.4	–	–	–	5.4	468	0.839	–	1.16	–	–
	12	100.4	10.7	–	–	–	5.32	463	0.903	–	1.16	–	–
	16	100.9	10	–	–	–	5.55	465	1.23	–	1.16	–	–
	18	101.1	11	–	–	–	5.36	466	1.22	–	1.25	–	–
	26	100.2	10.6	–	–	–	5.49	462	1.59	–	1.33	–	–
30 °C	0	N/A	11.1	–	–	–	5.53	461	–	–	1.33	–	–
pH 4.2	1	100	12.3	–	–	–	6.1	461	0.677	0.557	1.54	–	–
	2	100.7	12.2	–	–	–	5.36	464	0.598	–	1.34	–	–
	4	101.5	12.1	–	–	–	5.87	468	1.12	–	1.38	–	–
	8	100.9	11.9	–	–	–	5.7	465	2.06	–	1.13	0.632	–
	10	101.1	11	–	–	–	5.21	466	2.46	–	1.07	0.731	–
	12	99.3	11.4	–	–	–	5.26	458	2.92	–	1.12	0.869	–
	16	99.6	12.5	–	–	–	5.47	459	3.95	–	1.09	1.17	–
	18	99.1	13.1	–	–	–	5.13	457	4.56	–	1.17	1.42	–
	26	98	13	–	–	–	5.31	452	6.28	–	1.15	1.74	–
40 °C	0	N/A	11.1	–	–	–	5.53	461	–	–	1.33	–	–
pH 4.2	1	99.8	11.2	–	–	–	6.17	460	1.45	0.737	1.84	–	–
	2	100.4	12.6	–	–	–	5.45	463	1.61	–	1.45	0.537	–
	4	99.6	11.9	–	–	–	5.82	459	3.51	–	1.47	1.09	–
	8	98.5	14.5	–	–	–	5.58	454	6.49	–	1.12	1.87	–
	10	97.4	14.1	–	–	–	5.06	449	7.26	–	1.08	1.99	–
	12	96.5	15.2	–	–	–	5.07	445	8.54	–	1.2	2.39	–
	16	95.7	16.8	–	–	–	5.19	441	12.1	–	1.18	3.39	–
	18	95	17.7	–	0.595	–	5.03	438	12.6	–	1.27	3.61	–
	26	92.2	19.7	–	1.13	–	4.96	425	17.60	–	1.14	5	–

A typical HPLC chromatogram for the presence of steviol glycosides at 20 °C of pH 2.8 for week-12 is shown in **Fig. 4.**

The rate of degradation product formation was increased at lower pH levels and at higher temperatures. The majority of degradation product formation occurred after extended storage.

The pattern of Rebaudioside M (6) degradation was similar at each of the conditions tested although the extent and rate of degradation product formation were pH, temperature, and time-dependent.

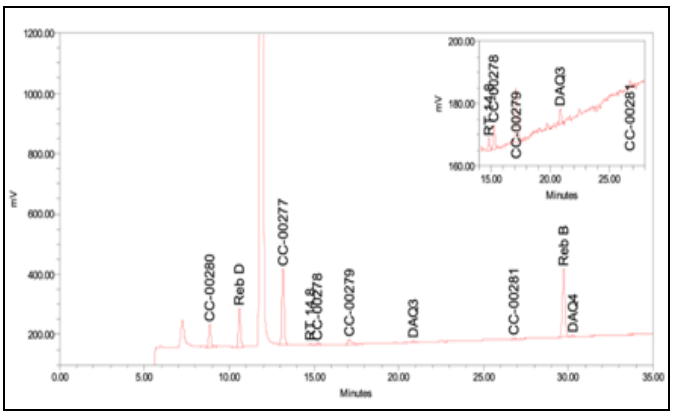

FIG. 4: TYPICAL HPLC CHROMATOGRAM

There was minimal degradation of the Rebaudioside M by exposure to a minimum of 1.2 million lux hours of fluorescent light and then to not less than 200-watt hours/m^2 of near ultra-violet light at 25 °C. The test/control samples total molar ratio was 100.0% as shown in **Tables 5** and **6.**

TABLE 5: SUMMARY OF PH 3.8 LIGHT TREATED SAMPLES (mg/L)

	Compounds					
Condition	**1**	**5**	**6**	**7**	**8**	**9**
Fluorescent Light	13.8	6.37	462	0.9	0.537	1.33
Control Fluorescent Light	11.9	5.29	467	0.737	-	1.16

TABLE 6: CALCULATED MOLAR EQUIVALENTS AT PH 3.8 LIGHT TREATED SAMPLE (MICRO MOLE/L)

	Compounds							
Condition	**1**	**5**	**6**	**7**	**8**	**9**	**Total**	**Fluorescent Light vs Control (%)**
Fluorescent Light	1.71	5.64	358	0.697	0.426	1.04	383	100
Control Fluorescent Light	14.8	4.68	362	0.571	-	0.91	383	NA

In this study, samples prepared at pH 3.2 and stored for 12 weeks at 20 °C represent conditions that are more stringent than the recognized conditions for evaluating non-nutritive sweeteners stability in carbonated soft drinks. After 12 weeks of storage at 20 °C, Rebaudioside M in the pH 3.2 samples was quantitated at 98.5% of initial level. In addition to Rebaudioside M, five other known degradation products or impurities (1, 5, 7, 9 and 10) were detected at levels greater than or equal to 0.500 mg/L (0.100%). All known degradation products or impurities detected at 12 weeks were present at 0 week except 10. The compound 10 is a known steviol glycoside. After 12 weeks at 20 °C, pH 3.2, 10 and 7 had the greatest increase in concentrations when compared to their initial 0-week levels but are minor constituents of the overall formulation (~500 mg/L). The compound 10 and 7 increased from <0.500 to 1.47 mg/L and 0.587 to 5.31 mg/L, respectively. No other known degradation products or impurities increased by more than fifteen percent of their initial 0-week levels under standard conditions. The results obtained in this study by LC-MS/MS confirm the chemical identities of the five compounds listed above as impurities and/or degradation products of Rebaudioside Min mock beverage under relevant conditions of use.

After 26 weeks of storage at 20 °C, the Rebaudioside Min the pH 3.2 samples was quantitated at 96.8% of initial level. In addition to Rebaudioside M, five other known degradation products or impurities (1, 5, 7, 9 and 10) were detected at levels greater than or equal to 0.500 mg/L (0.100%). All known degradation products or impurities detected at 26 weeks were present at 0 week except 10. The compound 10 is a known steviol glycoside. After 26 weeks at 20 °C, pH 3.2,10 and 7 had the greatest increase in concentrations when compared to their initial 0-week levels but are minor constituents of the overall formulation (~500 mg/L). The compound 10 and 7 increased from <0.500 to 3.06 mg/L and 0.587 to11.0 mg/L, respectively. No other known degradation products or impurities increased by more than fifteen percent of their initial 0-week levels under standard conditions.

The stability of Rebaudioside Min mock beverage solutions was pH-, temperature-, and time-dependent. The rate of degradation product formation was increased at lower pH levels and at higher temperatures. The majority of degradation product formation occurred after extended storage. After 12 weeks of storage, concentrations of Rebaudioside Min samples at pH 2.8, relative to the initial concentration, ranged from 99.8% at 5 °C to 61.3% at 40 °C. Similarly, at pH 3.2, concentrations of Rebaudioside Mranged from 100.0% at 5 °C to 77.6% at 40 °C; at pH 3.8, concentrations ranged from 100.0 at 5 °C to 92.7% at 40 °C; at pH 4.2, concentrations ranged from 100.2% at 5 °C to 96.5% at 40 °C.

After 26 weeks of storage, concentrations of Rebaudioside Min samples at pH 2.8, relative to the initial concentration, ranged from 99.4% at 5 °C to 34.1% at 40°C. Similarly, at pH 3.2, concentrations of Rebaudioside Mranged from 100.2% at 5 °C to 57.1%; at 40 °C; at pH 3.8, concentrations ranged from 99.6% at 5 to 83.4% at 40 °C; at pH 4.2, concentrations ranged from 100.9% at 5°C to 92.2% at 40°C. Patterns of Rebaudioside Mdegradation was similar at each of the conditions

tested although the extent and rate of degradation product formation were pH-, temperature, and time- dependent. The compound 1, 7, and 10 were the major degradation products detected during the study and were detected at increased concentration levels at every pH at 30°C and 40 °C. Lower pH levels caused greater formation of compounds 2, 3, and 11 than higher pH levels. The compound 5, 8, and 9 concentrations did not fluctuate throughout the study indicating that they are impurities and not degradation products.

The mole equivalents calculated from the analytical values of the analytes found in the mock beverages and their corresponding mass balances (Data not shown). The pH 3.2 samples stored at 20 °C for 12 weeks had a mass balance of 99.6 mole percent. The mass balance for all the other pH and storage conditions found after 12 weeks ranged from 97.7 to 100.4 mole percent. The pH 3.2 samples stored at 20 °C for 26 weeks had a mass balance of 99.7 mole percent. The mass balance for all the other pH and storage conditions found after 26 weeks of storage ranged from 97.2 mole percent to 100.4 mole percent. The high values found for molar recoveries in this study make it unlikely that any appreciable amount of an undetected degradation product was formed under the conditions of the study.

Appearance did not change throughout the study. All samples under all conditions were described as colorless, clear liquids, with no observed precipitates and many tiny white fibers. The LC-MS/MS confirmations are presented in **Table 7.**

TABLE 7: LC-MS/MS CONFIRMATION

	Compounds									
Analytical Detection Source	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**
Mock Beverage Results (mg/L) pH 2.8 5°C - Week 0										
CAD	11.1	-	-	-	5.5	-	-	1.12	-	-
LC-MS/MS	10.1a	-	-a	-a	7.24a	3.54	b	b	0.715	-
Mock Beverage Results (mg/L) pH 3.2 5°C - Week 0										
CAD	11.5	-	-	-	5.56	0.587	-	1.34	-	-
LC-MS/MS	10.1a	-	-a	-a	7.27a	1.98	b	b	-	-
Mock Beverage Results (mg/L) pH 2.8 40°C - Week 12										
CAD	27.4	2.5	8.96	-	3.12	95.9	-	0.737	29	2.03
LC-MS/MS	24.5a	2.87	9.19a	-a	4.12a	108	b	b	36.2	2.02
Mock Beverage Results (mg/L) pH 3.2 20°C - Week 12										
CAD	11.2	-	-	-	5.2	5.31	-	1.2	1.47	-
LC-MS/MS	10.3a	-	-a	-a	6.86a	7.19	b	b	1.77	-

a - 5, 1, 3 and 4 are estimate as the reference standard used in the preparation of standard solution had expired. b - due to limited reference materials for 8 and 9 only peak identification was performed. Below limit of quantification

Under relevant conditions of intended use (pH 3.2, 20 °C for 12 weeks), five compounds (1, 5, 7, 9, and 10) were present at levels greater than or equal to 0.500 mg/L (0.100%) as determined by HPLC analysis. LC- MS/MS analysis confirmed the identity of the analytes.

LC-MS/MS analysis also confirmed that 1, 5, 7, and 9 were present at the time of mock beverage sample preparation. The compound 10 was not detected by HPLC at the time of mock beverage sample preparation, for all pH levels, but was detected in the LC-MS/MS analysis of the week-0, pH 2.8 mock beverage sample preparation. The amount detected was minimal (0.715 mg/L). The LC-MS/MS analysis was not performed at the study start, it was performed after the completion of the 26 weeks of storage; therefore, the minimal amount of 10 in the week-0, pH 2.8 sample was the result of degradation as 10 did increase throughout the 26-week course of the study based on the HPLC and LC-MS/MS analysis.

The concentrations of 8 and 9 by LC-MS/MS samples were not reported. Due to limited reference material quantitative standards could not be prepared, only peak identification could be performed. Stability of 8 and 9 reference materials has not been established. Based on the molecular weights and mass spectra the reference materials were acceptable for peak identification.

Rebaudioside M (6) has similar stability as that of Rebaudioside A (1) in both low and high pH applications. In heat-processed beverages, such as flavored ice-tea, juices, sport drinks, flavored milk, drinking yogurt and non-acidified teas, the sweetener shows good stability during High Temperature Short Time heat processing and on subsequent product storage. Appearance did not change throughout the course of the study. All samples under all conditions were described as colorless, clear liquids, with no observed precipitates and any tiny white fibers. The pH levels of all solutions were on target as the study progressed. Rebaudioside M samples underwent sensory evaluation in two batches: at low temperature (4° C) and at ambient temperature (~22° C). At either temperature condition, we found no difference in any evaluated sensory condition by Rebaudioside M source. Sweet and Sweet Aftertaste ratings increased from 100ppm to 300ppm at both temperatures, which was expected. Bitter, Drying Mouthfeel, and Bitter Aftertaste ratings did not differ significantly under any conditions, and participants did not give significant ratings of Sour and Sour Aftertaste for any sample. This suggests that the source of the Rebaudioside M samples in this test did not significantly attribute to the sensory features evaluated here.

TABLE 8: MEANS TABLE OF EVALUATED SENSORY FEATURES FOR REBAUDIOSIDE M SAMPLES AT 100 AND 300 PPM AT LOW TEMPERATURE (4°C). A'S AND B'S REPRESENT DIFFERENT GROUPING BASED ON POST-HOC TUKEY'S HSD (P < 0.05)

Attribute	100ppm			300ppm			HSD (95%)
	Leaf (PureCircle™)	Bioconverted (PureCircle™)	Fermented (Avansya)	Leaf (PureCircle™)	Bioconverted (PureCircle™)	Fermented (Avansya)	
Sweet Taste	4.9 B	4.7 B	4.4 B	8.2 A	8.5 A	8.1 A	1.2
Bitter Taste	1.7	1.9	1.9	2.2	2.3	2.2	NSD
Sour Taste	0	0.1	0.1	0.1	0.1	0.1	NSD
Drying Mouthfeel	3.8	4.2	3.9	4.2	4.4	4.2	NSD
Sweet Aftertaste	1.6 B	1.4 B	1.5 B	3.2 A	3.3 A	3.4 A	0.9
Bitter Aftertaste	0.7	0.6	0.7	0.9	1	0.7	NSD
Sour Aftertaste	0	0	0	0	0	0	NSD

TABLE 9: MEANS TABLE OF EVALUATED SENSORY FEATURES FOR REBAUDIOSIDE M SAMPLES AT 100 AND 300 PPM AT AMBIENT TEMPERATURE (~22° C). A'S AND B'S REPRESENT DIFFERENT GROUPING BASED ON POST-HOC TUKEY'S HSD (P < 0.05)

Attribute	100ppm			300ppm			HSD (95%)
	Leaf (PureCircle™)	Bioconverted (PureCircle™)	Fermented (Avansya)	Leaf (PureCircle™)	Bioconverted (PureCircle™)	Fermented (Avansya)	
Sweet Taste	5.1 B	5.1 B	4.6 B	8.5 A	8.5 A	8.8 A	1.2
Bitter Taste	2	2	2	2.1	2.7	2.3	NSD
Sour Taste	0.1	0	0	0	0.1	0.1	NSD
Drying Mouthfeel	4.2	4	3.9	4.1	4.1	4.5	NSD
Sweet Aftertaste	1.8 B	1.8 B	1.7 B	3.5 A	3.3 A	3.6 A	0.9
Bitter Aftertaste	0.8	0.8	0.7	0.8	0.9	0.8	NSD
Sour Aftertaste	0.1	0	0	0	0.1	0	NSD

CONCLUSION: The stability of Rebaudioside Min mock beverage solutions is pH-, temperature-, and time- dependent. The rate and extent of degradation product formation is increased under acidic conditions (lower pH) and at higher temperatures with the majority of degradation product formation occurring after extended storage. Excellent mass balance was achieved under all conditions. In addition to Rebaudioside Mfive other known degradation products or impurities (10, 5, 7, 9, and 1) were detected at levels greater than or equal to 0.500 mg/L (0.100%). The high values found for all molar recoveries in this study make it unlikely that any appreciable amount of undetected degradation products were formed under the conditions of the study. There was minimal

degradation of the Rebaudioside M when exposed to fluorescent light. Thus, Rebaudioside M in mock beverages under relevant conditions of intended use is considered stable. Also, rebaudioside M produced from extraction of Stevia leaf, bio-conversion of rebaudioside A or by fermentation of glucose have the same sensory profile.

ACKNOWLEDGEMENT: We thank Gil Ma and Covance Laboratories, Madison, WI, USA for their help in providing necessary technical support.

Contribution by Each Author:

- Indra Prakash
- Juvenal Higiro
- Laura Martin
- Lauren Seabrooks

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Mosettig E and Nes WR: Stevioside. II. The structure of the aglucon. Journal of Organic Chemistry 1955; 20.
2. Okonkwo CE, Adeyanju AA, Onyeaka,Nwonuma CO, Olaniran AF, Alejolowo, OO, Inyinbor AA, Oluyori AP and Zhou C: "A review on rebaudioside M: The next generation steviol glycoside and noncaloric sweetener". Journal of Food Science 2024; 89: 6946-6965.
3. Song, F, Shending W, Mao, Y, Yuan, M, Zheng Q, Zheng Q, Shuli L and Ying L: "Enhancing Rebaudioside M Synthesis *via* Introducing Sulfur-Mediated Interactions between Glycosyltransferase UGT76G1 and Rebaudioside D" Journal of Agricultural and Food Chemistry 2025; 73: 667-677.
4. Brandle JE, Starrratt AN and Gijen M: *Stevia rebaudiana*: its agricultural, biological and chemical properties. Canadian Journal of Plant Science 1998; 78: 527-536.
5. Prakash I and Chaturvedula V: Steviol Glycosides: Natural Non-Caloric Sweeteners in Sweeteners, Springer International Publishing Switzerland 2016; 1-28. Steinmetz WE and Lin A: NMR studies of the conformation of the natural sweetener rebaudioside A. Carbohydr Res 2009; 344(18): 2533-8.
6. Lewis WH: Early uses of *Stevia rebaudiana* (Asteraceae) leaves as a sweetener in Paraguay. Economic Botany 1992.
7. Prakash I, Markosyan A and Bunders C: Development of Next Generation Stevia Sweetener: Rebaudioside M. Foods 2014; 3(1): 162-175.
8. Grant ED, Rafael ISM, Carr BT, Wilkens K, Bechman A and Prakash I: "The Concentration/Response Function Behavior of Non-caloric and Caloric Sweeteners". International Journal of Pharmaceutical Sciences and Research 2024; 15: 2289-2295.
9. Kinghorn ADK and Kim NC: Terpenoid glycoside sweeteners, in Naturally Occurring Glycosides, R. Ikan, Editor. John Wiley & Sons: New York, NY, USA 1999; 399-429.
10. Kinghorn ADW, CD and Soejarto DD: Stevioside, in Alternative Sweeteners, G. O'Brien Nabors & L., R.C, Editor. Marcel Dekker: New York, NY, USA 2001; 167-183.
11. Carakostas MP, Prakash I, Kinghorn AD, Wu CD and Soejarto DD: Steviol glycoside. In Alternative Sweeteners. 4th ed, ed. L. O'Brien Nabors, Ed. New York, NY, USA: Marcel Dekker 2012.
12. Chaturvedula V, Clos J and Prakash I: Stability of Steviol Glycosides in Mock Beverages Under Acidic Conditions. Int J Pharm Pharm Sci 2011; 3.
13. Chaturvedula V, Upreti M and Prakash I: Diterpene Glycosides from *Stevia rebaudiana*. Molecules (Basel, Switzerland) 2011; 16: 3552-62.
14. Chaturvedula V and Prakash I: A new diterpene glycoside from *Stevia rebaudiana*. Molecules 2011; 16(4): 2937-43.
15. Chaturvedula V and Prakash I: Structures of the novel diterpene glycosides from *Stevia rebaudiana*. Carbohydr Res 2011; 346(8): 1057-60.
16. Chaturvedula VS: Two minor diterpene glycosides from the leaves of *Stevia rebaudiana*. Nat Prod Commun 2011; 6(2): 175-8.
17. Chaturvedula V and Prakash I: Additional minor diterpene glycosides from *Stevia rebaudiana*. Nat Prod Commun 2011; 6(8): 1059-62.
18. Chaturvedula VS, Upreti M and Prakash I: Diterpene glycosides from *Stevia rebaudiana*. Molecules 2011; 16(5): 3552-62.
19. Kinghorn AD and Soejarto DD: Intensely sweet compounds of natural origin. Med Res Rev 1989; 9(1): 91-115.
20. Kinghorn AD and Less CM: Common high-potency sweeteners, in Alternative Sweeteners, G. O'Brien Nabors & L., R.C, Editor. Marcel Dekker: New York, NY, USA 1991; 197-218.
21. Kohda H: New sweet diterpene glucosides from *Stevia rebaudiana*. Phytochemistry 1976; 15(6): 981-983.
22. Prakash I: Development of rebiana, a natural, non-caloric sweetener. Food Chem Toxicol 2008; 46(7): 75-82.
23. Ohta M: Characterization of Novel Steviol Glycosides from Leaves of *Stevia rebaudiana* Morita. Journal of Applied Glycoscience 2010; 57(3): 199-209.
24. Prakash I: Synthesis and sensory evaluation of ent-kaurane diterpene glycosides. Molecules 2012; 17(8): 8908-16.
25. Prakash I, Campbell M and Chaturvedula V: Catalytic hydrogenation of the sweet principles of *Stevia rebaudiana*, Rebaudioside B, Rebaudioside C, and Rebaudioside D and sensory evaluation of their reduced derivatives. Int J Mol Sci 2012; 13(11): 15126-36.
26. Prakash I, Chaturvedula V and Markosyan A: Isolation, Characterization and Sensory Evaluation of a Hexa beta-D-Glucopyranosyl Diterpene from *Stevia rebaudiana*. Natural Product Communications 2013; 8: 1523-6.
27. Prakash I, Markosyan A, Chaturvedula V, Campbell M, San Miguel R, Purkayastha S and Johnson M: Methods for Purifying Steviol Glycosides and Uses of the Same 2013.
28. Chaturvedula V, Clos JF & Prakash I: Stability study of steviol glyco- sides in mock beverages using fluorescent light exposure under ICH guidelines. In-ternational Journal of Pharmacy and Pharmaceutical Sciences 2011; 3: 316-323.
29. Clos JF, DuBois GE and Prakash I: Photostability of Rebaudioside A and Stevioside in Beverages. Journal of Agricultural and Food Chemistry 2008; 56(18): 8507-8513.

30. Kroyer G: Stevioside and Stevia-sweetener in food: application, stability and interaction with food ingredients. Journal für Verbraucherschutz und Lebensmittelsicherheit 2010; 5(2): 225-229.
31. Catharino R and Santos L: Stevioside sweetener hydrolysis to steviol in acidic aqueous solutions. Food Chemistry 2012; 133.
32. Wölwer-Rieck U, Tomberg W and Wawrzun A: Investigations on the Stability of Stevioside and Rebaudioside A in Soft Drinks. Journal of Agricultural and Food Chemistry 2010; 58(23): 12216-12220.
33. Prakash I, Markosyan A, Chaturvedula V, Campbell M, Miguel RS, Purkayastha S and Johnson M: Methods for purifying Steviol Glycosides and uses of the same US patent 9: 169285.
34. Markosyan A, Jarin C, Robe P, Halle R, Prakash I and Chaturvedula V: Methods for making rebaudisoide XUS patent 9: 243-273.

How to cite this article:
Prakash I, Higiro J, Martin L and Seabrook L: Stability of rebaudioside m under acidic conditions and its degradation products. Int J Pharm Sci & Res 2025; 16(10): 2739-52. doi: 10.13040/IJPSR.0975-8232.16(10).2739-52.