


APPENDIX D

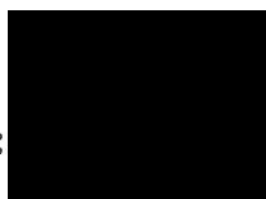
Study Reports for the Stability of Steviol Glycoside Mixtures

 PureCircle Sdn Bhd PureCircle pH stability of A95	Report# A95_pH_Stability	Issue date: 16/10/15
		Revision date: -----



pH Stability of A95

Prepared by :



(Process Scientist)


19/10/15
Date

Approved by :



(QA/QC Manager)

19/10/15
Date

 PureCircle Sdn Bhd PureCircle pH stability of A95	Report# A95_pH_Stability	Issue date: 16/10/15
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Objective


To determine pH stability under different temperature of A95 produced by PureCircle.
The pH stability of A95 will be studied by understanding the changes of the Total Steviol Glycosides (TSG).

Sample

One sample representing commercial lot of A95 labeled as “PT21052015”.

Standards

1. Rebaudioside A Standard (USP Rockville, MD USA, Lot#F0I077), or equivalent;
2. Stevioside Standard (USP Rockville, MD USA, Lot#F0I080), or equivalent;
3. Rebaudioside B Standard (Chromadex Inc. Irvine, CA USA, Lot#00018237-001), or equivalent.
4. Rebaudioside C Standard (Chromadex Inc. Irvine, CA USA, Lot#00018228-3962), or equivalent;
5. Rebaudioside D Standard (PureCircle Sdn. Bhd., Malaysia, Lot#L03082015), or equivalent;
6. Rebaudioside F Standard (Chromadex Inc. Irvine, CA USA, Lot#00018305-103), or equivalent;
7. Dulcoside A Standard (Chromadex Inc. Irvine, CA USA, Lot#04949-002), or equivalent;
8. Rubusoside Standard, (PureCircle Sdn. Bhd., Malaysia, Lot#270308), or equivalent;
9. Steviolbioside Standard (Chromadex Inc. Irvine, CA USA, Lot#00019349-1821), or equivalent.
10. Rebaudioside M Standard (PureCircle Sdn. Bhd., Malaysia, Lot#L11112013), or equivalent;

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11. Rebaudioside E Standard (Chromadex Inc. Irvine, CA USA, Lot#00018235-131), or equivalent.
12. Rebaudioside O Standard (Chromadex Inc. Irvine, CA USA, Lot#00018233-134), or equivalent.
13. Rebaudioside N Standard (Chromadex Inc. Irvine, CA USA, Lot#0018232-121), or equivalent.

Solvents and Reagents

1. Acetonitrile, HPLC grade (JT Baker, USA);
2. Water, HPLC grade (JT Baker, USA);
3. Sodium dihydrogen phosphate monohydrate, reagent grade (Merck, Germany);
4. Sodium hydrogen phosphate anhydrous, reagent grade (Fisher Scientific, USA);
5. Ortho-phosphoric acid 85%, reagent grade (SYSTEM, USA).

Apparatus

1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Poroshell 120 SB-C18, 4.6x150mm, 2.7 μ m (PN 683975-902), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Volumetric (class A) and Laboratory glassware.

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Preparation Sample Solution


Sample solution was prepared at approximately 50000 mg/L in 500 mL of diluent with pH vary from pH2-pH7. The buffer solutions were prepared by mixing different ratio of 0.1M phosphate buffer (Sodium dihydrogen phosphate monohydrate, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ Merck®, Germany), 0.1M phosphorous acid (*ortho*-Phosphoric Acid 85%, H_3PO_4 . System ®, ChemPur), or 0.1M Sodium hydrogen phosphate buffer (Sodium hydrogen phosphate anhydrous, Na_2HPO_4 , Fisher Scientific ®, USA)

Sample solution storage

Each pH level was stored at 4 different temperature conditions as 5°C, 25°C, 37°C and 56°C in amber glass bottles.

Analysis

Analysis and calculations were performed as per calculation section described in “HPLC Assay of A95 samples in 5 commercial lots, report # KKN02102015001” (APPENDIX A)

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Result

The assay results are summarized in Table 1-4. The Graph 1-7 show the dynamic changes of A95 in various pH solutions and various temperatures.

Table 1

A95 assay at 5°C

A95 and related steviol glycosides assay at 5°C							
Total steviol glycosides, (mg/ml)							
weeks	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
0	859.68	861.32	869.66	874.61	856.27	862.88	867.45
1	872.25	871.65	880.50	877.88	875.30	875.55	879.31
2	859.80	873.47	880.81	868.44	871.32	875.02	870.11
4	863.33	871.08	877.61	875.86	871.05	877.16	877.64
8	735.33	852.57	861.67	851.21	849.91	862.23	855.72
13	751.00	874.57	880.90	881.32	868.70	888.32	887.98
17	787.35	889.27	883.69	875.98	903.55	880.12	883.35

Table 2

A95 assay at 25°C

A95 and related steviol glycosides assay at 25°C							
Total steviol glycosides, (mg/ml)							
weeks	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
0	859.68	861.32	869.66	874.61	856.27	862.88	867.45
1	828.10	865.52	871.28	871.49	863.32	877.90	871.77
2	802.56	858.55	879.90	875.98	874.24	872.40	882.36
4	738.87	864.92	876.93	869.46	864.87	874.71	873.74
8	555.23	849.37	865.04	852.03	842.03	854.68	852.61
13	548.53	869.53	886.87	882.10	861.44	847.99	872.01
17	463.40	872.26	902.60	891.69	887.03	832.34	873.90


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Table 3


A95 assay at 37°C

A95 and related steviol glycosides assay at 37°C							
Total steviol glycosides, (mg/ml)							
weeks	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
0	859.68	861.32	869.66	874.61	856.27	862.88	867.45
1	623.94	859.80	875.30	872.13	875.19	882.15	881.07
2	462.78	863.97	877.76	859.77	863.46	875.79	869.52
4	284.12	851.43	871.24	865.44	867.09	872.54	870.47
8	110.25	807.55	851.01	836.91	847.75	852.62	853.15
13	58.60	812.54	868.78	860.38	869.02	874.35	877.79
17	44.99	801.24	869.80	852.18	875.04	864.32	879.09

Table 4

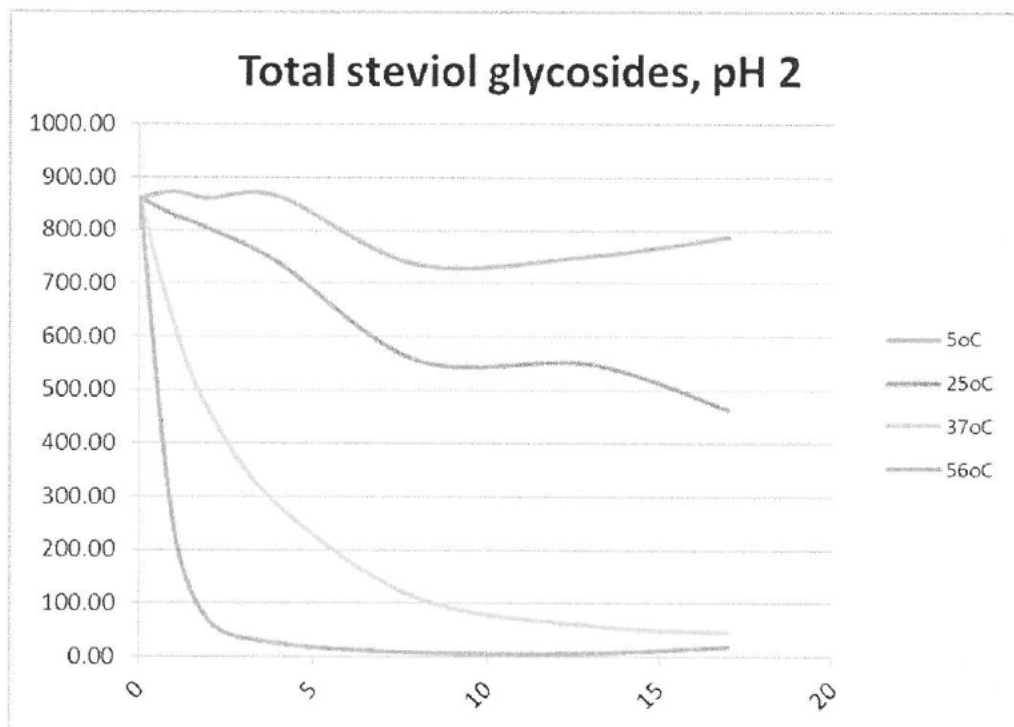
A95 assay at 56°C

A95 and related steviol glycosides assay at 56°C							
Total steviol glycosides, (mg/ml)							
weeks	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
0	859.68	861.32	869.66	874.61	856.27	862.88	867.45
1	241.75	850.64	877.68	881.68	873.82	877.55	873.80
2	68.68	809.88	874.56	871.89	861.12	848.41	839.36
4	25.17	724.92	850.00	855.10	834.73	768.72	814.09
8	7.95	552.33	817.44	822.10	691.29	664.74	758.27
13	6.64	438.45	795.56	803.29	518.95	579.60	668.54
17	18.58	163.83	804.52	729.90	444.48	535.36	626.21

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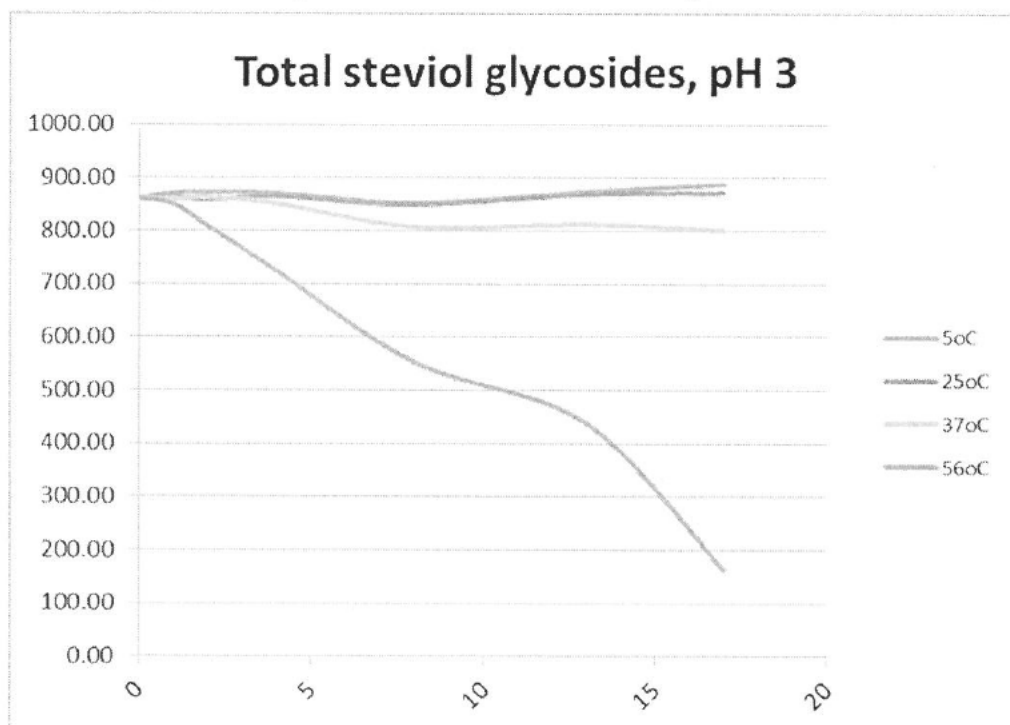
Graph 1


TSG changes of A95 pH2 solution in different temperatures



Graph 2

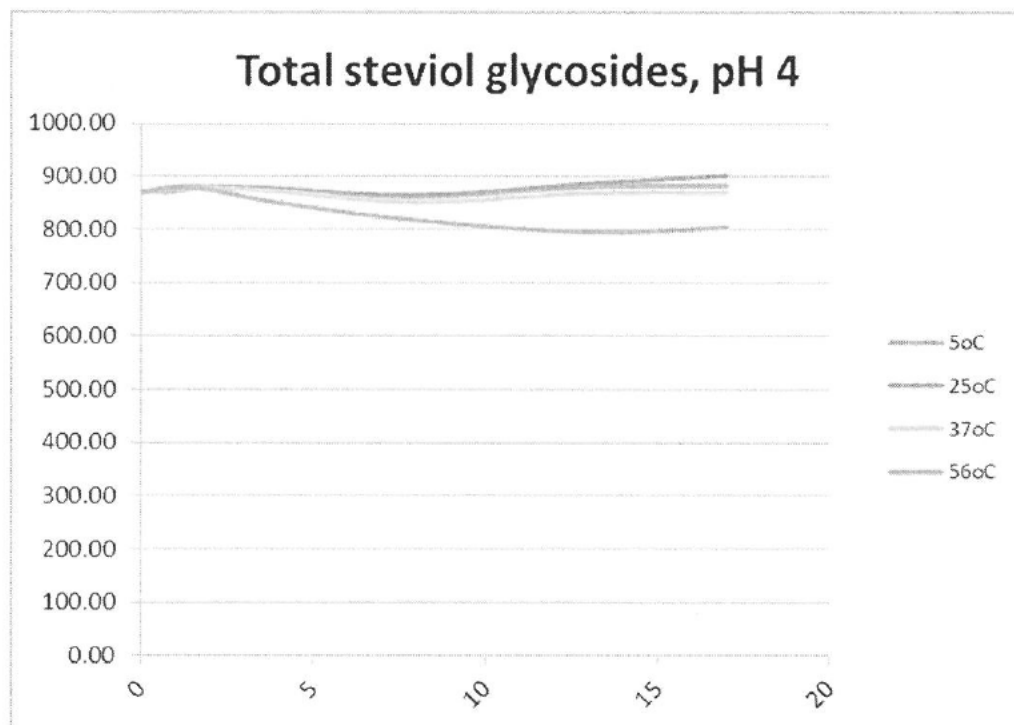
TSG changes of A95 pH3 solution in different temperatures



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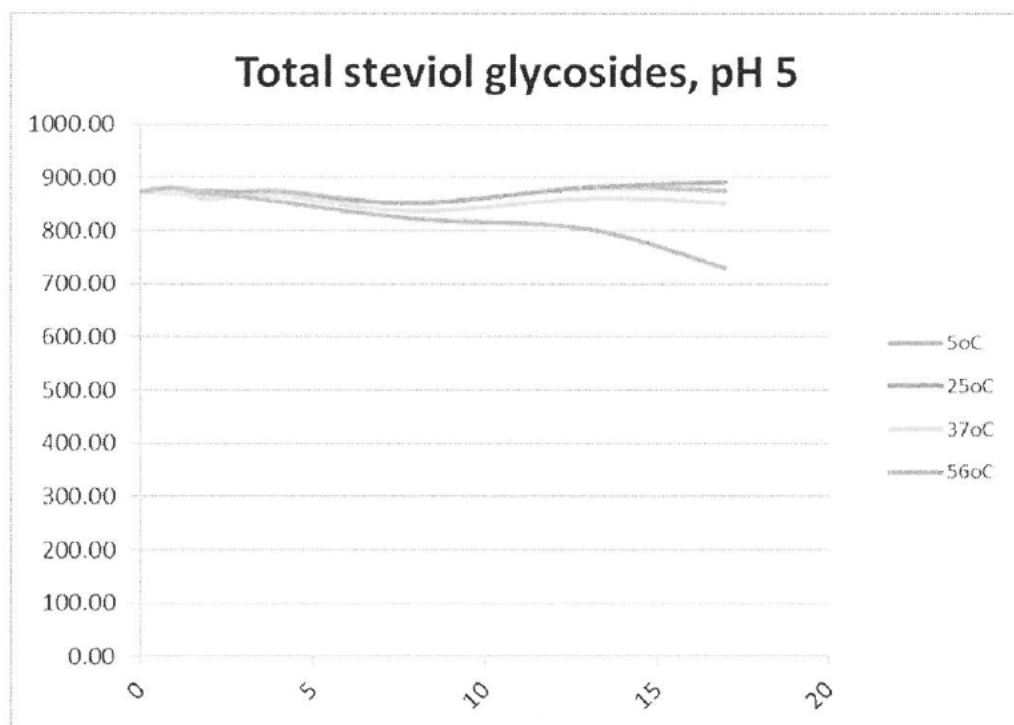
Graph 3


TSG changes of A95 pH4 solution in different temperatures



Graph 4

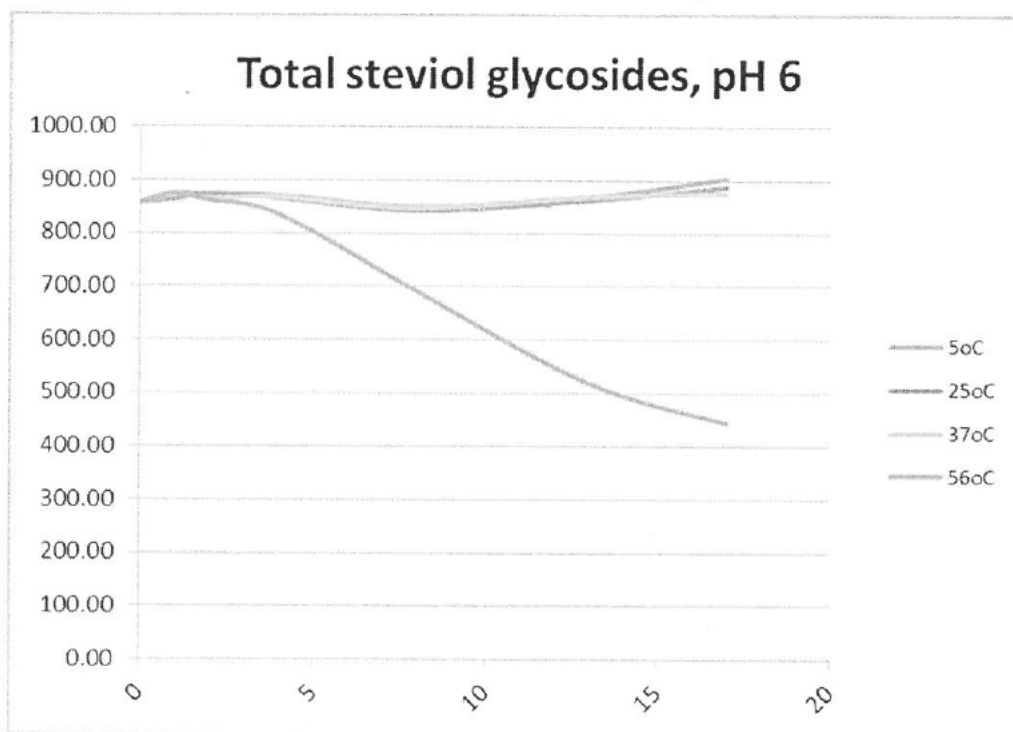
TSG changes of A95 pH5 solution in different temperatures



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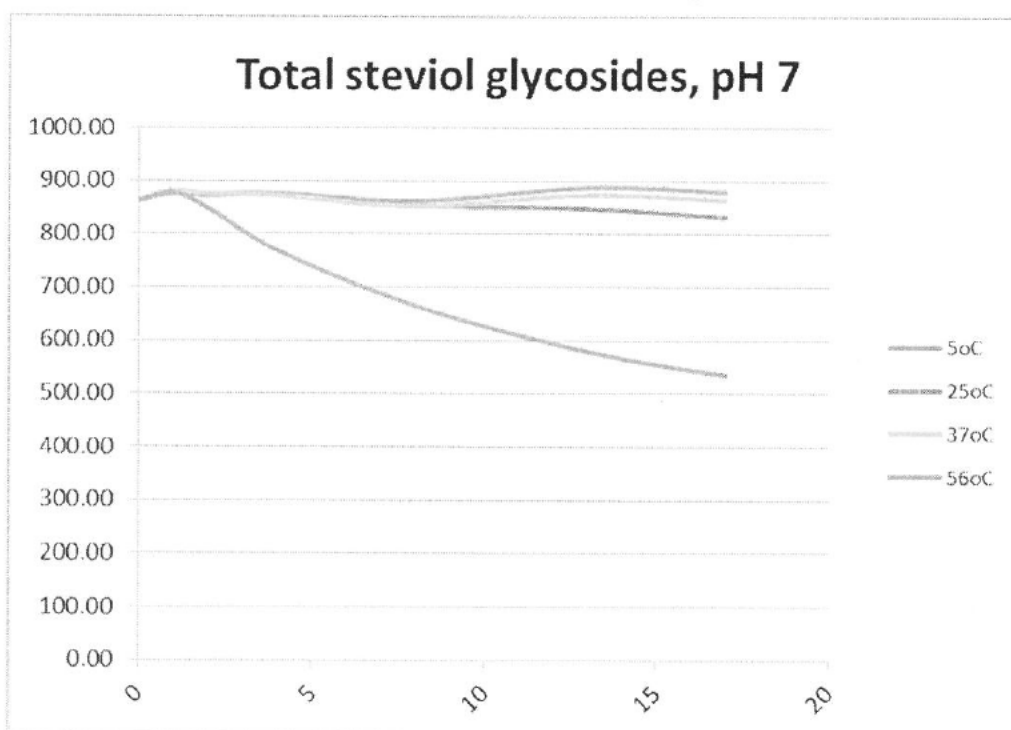
Graph 5


TSG changes of A95 pH6 solution in different temperatures



Graph 6

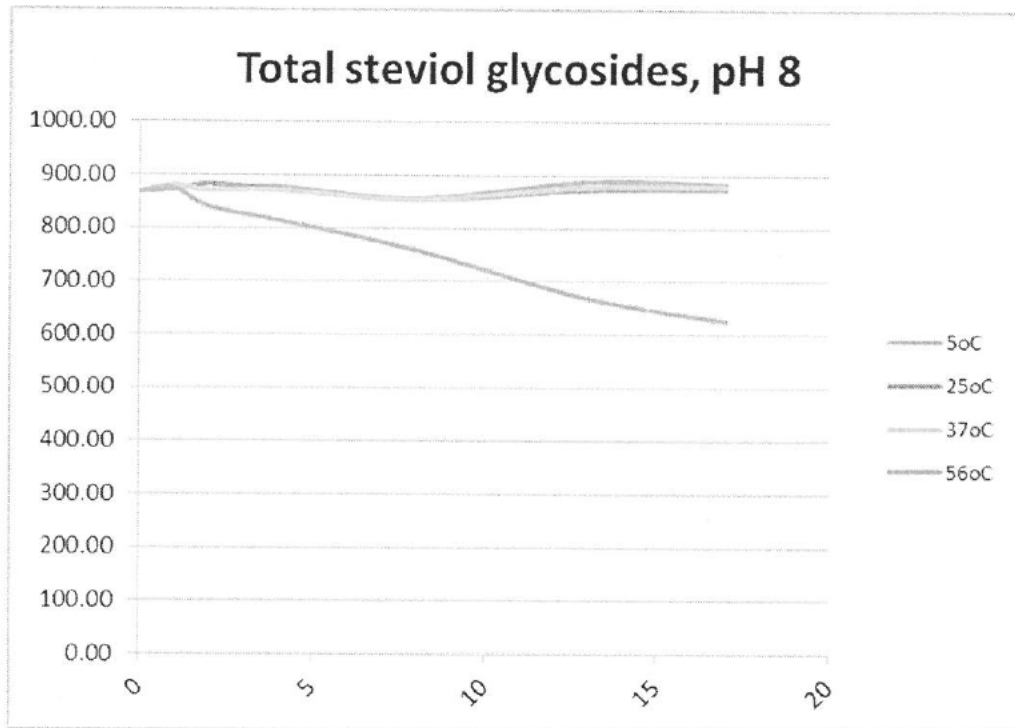
TSG changes of A95 pH7 solution in different temperatures




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Graph 7


TSG changes of A95 pH8 solution in different temperatures



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APPENDIX A

Calculation section extracted from “**HPLC Assay of A95 samples in 5 commercial lots,**
report # KKN02102015001”

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Calculation

Rebaudioside D : Prepare a standard curve for rebaudioside D by plotting rebaudioside D peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_D) of rebaudioside D in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside D in the portion of the sample taken by the formula:

$$C_D / C_{SMP} \times 100 \%$$

C_D = Concentration of rebaudioside D in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Rebaudioside M : Prepare a standard curve for rebaudioside M by plotting rebaudioside M peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_M) of rebaudioside M in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside M in the portion of the sample taken by the formula:


$$C_M / C_{SMP} \times 100 \%$$

C_M = Concentration of rebaudioside M in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Rebaudioside A : Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_A) of rebaudioside A in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside A in the portion of the sample taken by the formula:

$$C_A / C_{SMP} \times 100 \%$$

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C_A = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Stevioside : Prepare a standard curve for Stevioside by plotting Stevioside peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_S) of Stevioside in the *Sample solution* in mg/L. Calculate the content (%) of Stevioside in the portion of the sample taken by the formula:

$$C_S / C_{SMP} \times 100 \%$$

C_S = Concentration of Stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Other steviol glycosides: Rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusosodie and steviolbioside use the stevioside standard cuve prepared above to calculated the mg/L stevioside for each. Separately calculate the percents of each analyte (rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusoside and steviolbioside) in the sample taken using the following formula which takes into account the difference in molecular weights between the analytes and stevioside:


$$(C_S \times f_s) / C_{SMP} \times 100 \%$$

C_S = Concentration of Stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

f_s = ratio of the formula weight of others steviol glycosides to the formula weight of stevioside; 1.00 (rebaudioside B), 1.16 (rebaudioside F), 1.18 (rebaudioside C), 0.98 (dulcoside A), 0.80 (rubusoside), and 0.80 (steviolbioside)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Rebaudioside E, rebaudioside O and rebaudioside N use the rebaudioside D standard curve prepared above to calculate the mg/L rebaudioside D for each. Separately

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calculate the percents of each analyte (Rebaudioside E, rebaudioside O and rebaudioside N) in the sample taken using the following formula which takes into account the difference in molecular weights between the analytes and stevioside:

$$(C_D \times f_D) / C_{SMP} \times 100 \%$$

C_D = Concentration of Stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

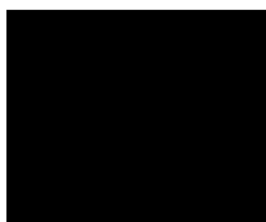
f_D = ratio of the formula weight of others steviol glycosides to the formula weight of Rebaudioside D; 0.86 (rebaudioside E), 1.27 (rebaudioside O) and 1.13 (rebaudioside N)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

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Storage Stability of A95 powder

Prepared by :



(Process Scientist)

23/10/15
Date


Approved by :



(QA/QC Manager)

23/10/15
Date



 PureCircle Sdn Bhd PureCircle Storage stability of A95 powder	Report# A95_Storage_Stability	Issue date: 23/10/15
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Objective

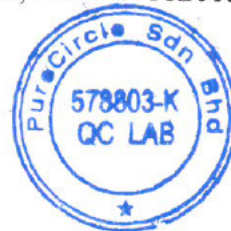
To determine storage stability under different temperature and different container of A95 powder produced by PureCircle. The storage stability of A95 powder will be studied by understanding the changes of the Total Steviol Glycosides (TSG).


Sample

One sample representing commercial lot of A95 labeled as "PT21052015".

Standards

1. Rebaudioside A Standard (USP Rockville, MD USA, Lot#F0I077), or equivalent;
2. Stevioside Standard (USP Rockville, MD USA, Lot#F0I080), or equivalent;
3. Rebaudioside B Standard (Chromadex Inc. Irvine, CA USA, Lot#00018237-001), or equivalent.
4. Rebaudioside C Standard (Chromadex Inc. Irvine, CA USA, Lot#00018228-3962), or equivalent;
5. Rebaudioside D Standard (PureCircle Sdn. Bhd., Malaysia, Lot#L03082015), or equivalent;
6. Rebaudioside F Standard (Chromadex Inc. Irvine, CA USA, Lot#00018305-103), or equivalent;
7. Dulcoside A Standard (Chromadex Inc. Irvine, CA USA, Lot#04949-002), or equivalent;
8. Rubusoside Standard, (PureCircle Sdn. Bhd., Malaysia, Lot#270308), or equivalent;
9. Steviolbioside Standard (Chromadex Inc. Irvine, CA USA, Lot#00019349-1821), or equivalent.
10. Rebaudioside M Standard (PureCircle Sdn. Bhd., Malaysia, Lot#L11112013), or equivalent;



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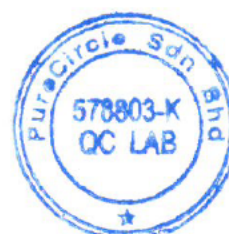
11. Rebaudioside E Standard (Chromadex Inc. Irvine, CA USA, Lot#00018235-131), or equivalent.
12. Rebaudioside O Standard (Chromadex Inc. Irvine, CA USA, Lot#00018233-134), or equivalent.
13. Rebaudioside N Standard (Chromadex Inc. Irvine, CA USA, Lot#0018232-121), or equivalent.


Solvents and Reagents

1. Acetonitrile, HPLC grade (JT Baker, USA);
2. Water, HPLC grade (JT Baker, USA);
3. Sodium dihydrogen phosphate monohydrate, reagent grade (Merck, Germany);
4. Ortho-phosphoric acid 85%, reagent grade (SYSTEM, USA).

Apparatus

1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Poroshell 120 SB-C18, 4.6x150mm, 2.7µm (PN 683975-902), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Volumetric (class A) and Laboratory glassware.



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Preparation of Sample

Total 100g of sample was packed in 2 amber glass containers and 2 aluminum bags, which is 25g each. 1 set of sample in glass container and aluminum bag was then placed in the 40°C, 75% humidity chamber while another set was stored under 25°C. The samples were withdrawn for the HPLC assay in a timely manner.

Analysis

Analysis and calculations were performed as per calculation section described in “HPLC Assay of A95 samples in 5 commercial lots, report # KKN02102015001” (APPENDIX A)



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Result

The assay results are summarized in Table 1-4.

Table 1

A95 assay of the powder sample kept in amber glass bottle, 25°C

A95 and related steviol glycosides assay at 25°C, amber glass bottle												
weeks	Reb E	Reb O	Reb D	Reb N	Reb M	Reb A	Stev	Reb F	Reb C	Dul A	Rub	TSG
0	0.89	1.39	66.48	3.00	25.38	1.37	0.04	0.01	0.02	0.00	0.00	98.75
1	0.86	1.42	65.61	2.92	24.30	1.35	0.03	0.01	0.01	0.00	0.00	96.69
4	0.84	1.38	65.38	2.99	25.27	1.36	0.04	0.01	0.01	0.00	0.00	97.44
8	0.89	1.39	68.30	3.00	23.56	1.39	0.04	0.00	0.01	0.00	0.00	98.77
13	0.87	1.36	66.47	2.97	23.83	1.37	0.03	0.00	0.02	0.00	0.00	97.09




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Table 2

A95 assay of the powder sample kept in aluminum, 25°C

B# PT21052015														
A95 and related steviol glycosides assay at 25°C, aluminium bag														
weeks	Reb E	Reb O	Reb D	Reb N	Reb M	Reb A	Stev	Reb F	Reb C	Dul A	Rub	Reb B	Sbio	TSG
0	0.89	1.39	66.48	3.00	25.38	1.37	0.04	0.01	0.02	0.00	0.00	0.17	0.00	98.75
1	0.84	1.37	64.78	2.84	25.01	1.33	0.04	0.01	0.01	0.00	0.00	0.17	0.00	96.41
4	0.85	1.36	65.20	2.93	25.29	1.34	0.04	0.01	0.01	0.00	0.00	0.18	0.00	97.20
8	0.88	1.35	66.61	3.01	22.74	1.36	0.05	0.00	0.00	0.00	0.00	0.19	0.00	96.20
13	0.87	1.39	67.21	2.99	23.29	1.37	0.03	0.00	0.00	0.00	0.00	0.21	0.00	97.36




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Table 3

A95 assay of the powder sample kept in amber glass bottle, 40°C, 75%RH

A95 and related steviol glycosides assay at 40°C, 75% RH, amber glass bottle												
weeks	Reb E	Reb O	Reb D	Reb N	Reb M	Reb A	Stev	Reb F	Reb C	Dul A	Rub	TSG
0	0.89	1.39	66.48	3.00	25.38	1.37	0.04	0.01	0.02	0.00	0.00	98.75
1	0.84	1.37	65.34	2.90	24.84	1.34	0.03	0.01	0.01	0.00	0.00	96.85
4	0.83	1.37	64.89	2.95	25.54	1.35	0.04	0.01	0.01	0.00	0.00	97.21
8	0.87	1.42	66.26	2.96	23.59	1.38	0.05	0.01	0.01	0.00	0.00	96.79
13	0.87	1.42	66.94	3.05	24.08	1.39	0.03	0.00	0.00	0.00	0.00	98.04



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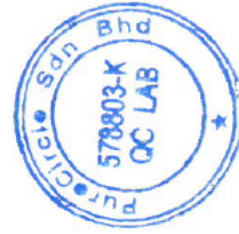
Storage stability of A95 powder




Table 4

A95 assay of the powder sample kept in aluminum bag, 40°C, 75%RH

A95 and related steviol glycosides assay at 40°C, 75% RH, aluminium bag												
weeks	Reb E	Reb O	Reb D	Reb N	Reb M	Reb A	Stev	Reb F	Reb C	Dul A	Rub	TSG
0	0.89	1.39	66.48	3.00	25.38	1.37	0.04	0.01	0.02	0.00	0.00	98.75
1	0.83	1.35	64.28	2.87	25.95	1.35	0.03	0.01	0.01	0.00	0.00	96.86
4	0.83	1.32	64.25	2.79	26.72	1.35	0.03	0.01	0.01	0.00	0.00	97.53
8	0.85	1.35	65.98	2.93	22.94	1.35	0.04	0.01	0.02	0.00	0.00	95.71
13	0.84	1.39	66.29	2.99	24.08	1.38	0.03	0.00	0.00	0.00	0.00	97.30




 PureCircle PureCircle Sdn Bhd	Report# A95_Storage_Stability	Issue date: 23/10/15
		Revision date: -----
Storage stability of A95 powder		Revision No: 00

APPENDIX A

Calculation section extracted from “**HPLC Assay of A95 samples in 5 commercial lots, report # KKN02102015001**”



 PureCircle Sdn Bhd Storage stability of A95 powder	Report# A95_Storage_Stability	Issue date: 23/10/15
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Calculation

Rebaudioside D : Prepare a standard curve for rebaudioside D by plotting rebaudioside D peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_D) of rebaudioside D in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside D in the portion of the sample taken by the formula:

$$C_D / C_{SMP} \times 100 \%$$

C_D = Concentration of rebaudioside D in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Rebaudioside M : Prepare a standard curve for rebaudioside M by plotting rebaudioside M peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_M) of rebaudioside M in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside M in the portion of the sample taken by the formula:

$$C_M / C_{SMP} \times 100 \%$$


C_M = Concentration of rebaudioside M in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Rebaudioside A : Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_A) of rebaudioside A in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside A in the portion of the sample taken by the formula:

$$C_A / C_{SMP} \times 100 \%$$



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C_A = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Stevioside : Prepare a standard curve for Stevioside by plotting Stevioside peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_S) of Stevioside in the *Sample solution* in mg/L. Calculate the content (%) of Stevioside in the portion of the sample taken by the formula:

$$C_S / C_{SMP} \times 100 \%$$

C_S = Concentration of Stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Other steviol glycosides: Rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusosodie and steviolbioside use the stevioside standard cuve prepared above to calculated the mg/L stevioside for each. Separately calculate the percents of each analyte (rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusoside and steviolbioside) in the sample taken using the following formula which takes into account the difference in molecular weights between the analytes and stevioside:

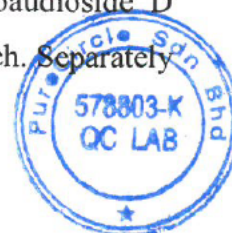
$$(C_S \times f_s) / C_{SMP} \times 100 \%$$


C_S = Concentration of Stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

f_s = ratio of the formula weight of others steviol glycosides to the formula weight of stevioside; 1.00 (rebaudioside B), 1.16 (rebaudioside F), 1.18 (rebaudioside C), 0.98 (dulcoside A), 0.80 (rubusoside), and 0.80 (steviolbioside)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Rebaudioside E, rebaudioside O and rebaudioside N use the rebaudioside D standard curve prepared above to calculate the mg/L rebaudioside D for each. Separately



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calculate the percents of each analyte (Rebaudioside E, rebaudioside O and rebaudioside N) in the sample taken using the following formula which takes into account the difference in molecular weights between the analytes and stevioside:


$$(C_D \times f_D) / C_{SMP} \times 100 \%$$

C_D = Concentration of Stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

f_D = ratio of the formula weight of others steviol glycosides to the formula weight of Rebaudioside D; 0.86 (rebaudioside E), 1.27 (rebaudioside O) and 1.13 (rebaudioside N)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)



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		Revision date: -----
pH stability of Reb A 50 powder		Revision No: 00

pH Stability of Reb A 50

Prepared by :

[Redacted Signature]

(QC Chemist)

27/10/2015
Date


Approved by :

[Redacted Signature]

(QA/QC Manager)

27/10/2015
Date



 PureCircle PureCircle Sdn Bhd	Report# RebA50_pH_Stability	Issue date: 27/10/15
		Revision date: -----
pH stability of Reb A 50 powder		Revision No: 00

Objective

To determine pH stability under different temperature of Reb A 50 produced by PureCircle. The pH stability of Reb A 50 will be studied by understanding the changes of the Total Steviol Glycosides (TSG).

Sample


One sample representing commercial lot of Reb A 50 labeled as "A0912266".

Standards

1. Rebaudioside A Standard (USP Rockville, MD USA, Lot#F0I077), or equivalent;
2. Stevioside Standard (USP Rockville, MD USA, Lot#F0I080), or equivalent;
3. Rebaudioside B Standard (Chromadex Inc. Irvine, CA USA, Lot#00018237-001), or equivalent.
4. Rebaudioside C Standard (Chromadex Inc. Irvine, CA USA, Lot#00018228-3962), or equivalent;
5. Rebaudioside D Standard (PureCircle Sdn. Bhd., Malaysia, Lot#L03082015), or equivalent;
6. Rebaudioside F Standard (Chromadex Inc. Irvine, CA USA, Lot#00018305-103), or equivalent;
7. Dulcoside A Standard (Chromadex Inc. Irvine, CA USA, Lot#04949-002), or equivalent;
8. Rubusoside Standard, (PureCircle Sdn. Bhd., Malaysia, Lot#270308), or equivalent;
9. Steviolbioside Standard (Chromadex Inc. Irvine, CA USA, Lot#00019349-1821), or equivalent.

Solvents and Reagents



 PureCircle Sdn Bhd pH stability of Reb A 50 powder	Report# RebA50_pH_Stability	Issue date: 27/10/15
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1. Acetonitrile, HPLC grade (JT Baker, USA);
2. Water, HPLC grade (JT Baker, USA);
3. Sodium dihydrogen phosphate monohydrate, reagent grade (Merck, Germany);
4. Ortho-phosphoric acid 85%, reagent grade (SYSTEM, USA).


Apparatus

1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Poroshell 120 SB-C18, 4.6x150mm, 2.7µm (PN 683975-902), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Volumetric (class A) and Laboratory glassware.
6. Sonicator

Preparation Sample Solution

Sample solution was prepared at approximately 1000 mg/L in 500 mL of diluent with pH vary from pH2-pH8. The buffer solutions were prepared by mixing different ratio of 0.1M phosphate buffer (Sodium dihydrogen phosphate monohydrate, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ Merck®, Germany), 0.1M phosphorous acid (*ortho*-Phosphoric Acid 85%, H_3PO_4 . Systemer®, ChemPur), or 0.1M Sodium hydrogen phosphate buffer (Sodium hydrogen phosphate anhydrous, Na_2HPO_4 , Fisher Scientific®, USA)



 PureCircle Sdn Bhd pH stability of Reb A 50 powder	Report# RebA50_pH_Stability	Issue date: 27/10/15
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Sample solution storage

Each pH level was stored at 4 different temperature conditions as 5°C, 25°C, 37°C and 56°C in amber glass bottles.

Analysis

Standard Curve Acceptance Criteria

The criteria of Standard Curve for rebaudioside A and Stevioside to be acceptable for use in the calibration curve, the standard recoveries must be within $100.0 \pm 3\%$ for all rebaudioside A and Stevioside concentration levels. No more than one standard may be omitted at each concentration level. The correlation coefficient for the standard curve is acceptable if it is greater than 0.9990.

Rebaudioside A: Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_A) of rebaudioside A in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside A in the portion of the sample taken by the formula:

$$C_A / C_{SMP} \times 100 \%$$


C_A = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Stevioside : Prepare a standard curve for Stevioside by plotting Stevioside peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_S) of Stevioside in the *Sample solution* in mg/L. Calculate the content (%) of Stevioside in the portion of the sample taken by the formula:

$$C_S / C_{SMP} \times 100 \%$$



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C_S = Concentration of Stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Other steviol glycosides: Rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusosodie and steviolbioside use the stevioside standard cuve prepared above to calculated the mg/L stevioside for each. Separately calculate the percents of each analyte (rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusoside and steviolbioside) in the sample taken using the following formula which takes into account the difference in molecular weights between the analytes and stevioside:


$$(C_S \times f_s) / C_{SMP} \times 100 \%$$

C_S = Concentration of Stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

f_s = ratio of the formula weight of others steviol glycosides to the formula weight of stevioside; 1.00 (rebaudioside B), 1.16 (rebaudioside F), 1.18 (rebaudioside C), 0.98 (dulcoside A), 0.80 (rubusoside), and 0.80 (steviolbioside)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)



 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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Result

The assay results are summarized in Table 1-4. The Graph 1-7 show the dynamic changes of Reb A 50 in various pH solutions and various temperatures.

Table 1

Reb A 50 assay at 5°C

5°+E5:L21C Total Steviol Glycosides, %							
Weeks	pH2	pH3	pH4	pH5	pH6	pH7	pH8
0	95.02	95.21	95.37	95.57	96.12	95.81	95.54
2	96.28	95.89	95.41	96.10	96.11	96.28	95.75
4	96.09	95.78	94.47	96.37	96.46	96.79	95.72
6	95.50	94.18	94.58	95.88	96.29	96.63	95.20
8	96.19	95.94	95.85	96.01	96.12	96.06	95.81
12	95.54	95.26	94.96	94.70	94.89	95.41	95.14
14	94.97	94.27	94.02	94.10	94.75	94.83	94.37
16	94.97	94.35	94.65	94.41	94.96	94.10	94.20
20	94.44	96.13	95.63	94.87	95.75	96.48	94.16
24	95.59	94.95	94.93	94.80	94.92	95.57	94.92
28	93.82	93.74	93.92	94.10	94.72	93.89	93.52
32	93.36	92.92	92.58	92.78	92.77	93.06	92.51
36	94.22	93.24	93.17	93.53	93.97	94.12	93.93
40	94.99	94.57	93.47	95.27	95.83	95.02	94.41
48	95.77	95.85	95.32	96.30	95.73	95.31	94.97




 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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Table 2

Reb A 50 assay at 25°C

25°C Total Steviol Glycosides, %							
Weeks	pH2	pH3	pH4	pH5	pH6	pH7	pH8
0	95.02	95.21	95.37	95.57	96.12	95.81	95.54
2	96.41	95.92	95.72	95.63	96.42	95.81	95.59
4	95.85	95.40	95.23	95.20	96.05	95.91	94.42
6	96.19	97.31	96.74	97.07	97.72	97.06	97.09
8	95.15	95.61	96.67	96.48	95.56	96.78	96.26
12	90.95	94.33	94.51	94.08	94.40	94.01	95.68
14	92.60	95.76	94.74	94.62	94.56	94.69	94.02
16	90.81	95.63	95.07	95.51	94.78	94.31	94.54
20	88.55	96.61	94.88	94.49	94.49	96.08	99.09
24	84.13	96.00	94.67	94.56	94.56	95.55	94.69
28	80.96	94.44	94.23	92.41	92.41	93.47	93.90
32	76.72	93.30	92.45	92.43	92.12	92.01	92.18
36	72.05	93.86	93.16	92.28	92.18	93.46	93.35
40	66.59	94.67	94.93	94.88	94.96	94.45	93.54
48	58.20	94.93	94.80	94.91	92.93	94.33	94.62




 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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Table 3

Reb A 50 assay at 37°C

37°C Total Steviol Glycosides, %							
Weeks	pH2	pH3	pH4	pH5	pH6	pH7	pH8
0	95.02	95.21	95.37	95.57	96.12	95.81	95.54
2	95.09	95.69	95.73	95.69	96.24	95.63	94.91
4	88.53	96.35	96.34	95.98	95.87	96.72	94.54
8	79.15	95.90	95.86	96.16	95.87	95.99	95.76
12	72.31	95.20	94.95	94.57	94.51	94.56	94.12
14	66.22	96.19	96.39	95.91	94.26	95.59	96.00
16	59.27	95.54	95.33	95.08	94.22	94.70	94.98
20	48.70	95.26	95.32	93.19	93.26	93.11	92.85
24	42.28	94.74	95.05	94.17	92.98	92.68	93.15
28	36.45	93.38	95.00	93.25	91.39	90.82	92.08
32	28.29	91.39	92.86	93.18	89.35	89.73	91.46
36	23.59	90.16	92.04	91.59	89.61	87.94	90.36
40	16.90	89.51	93.27	90.90	86.70	84.90	90.69
48	12.15	88.45	94.81	92.05	89.46	85.36	90.76





 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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Table 4

Reb A 50 assay at 56°C

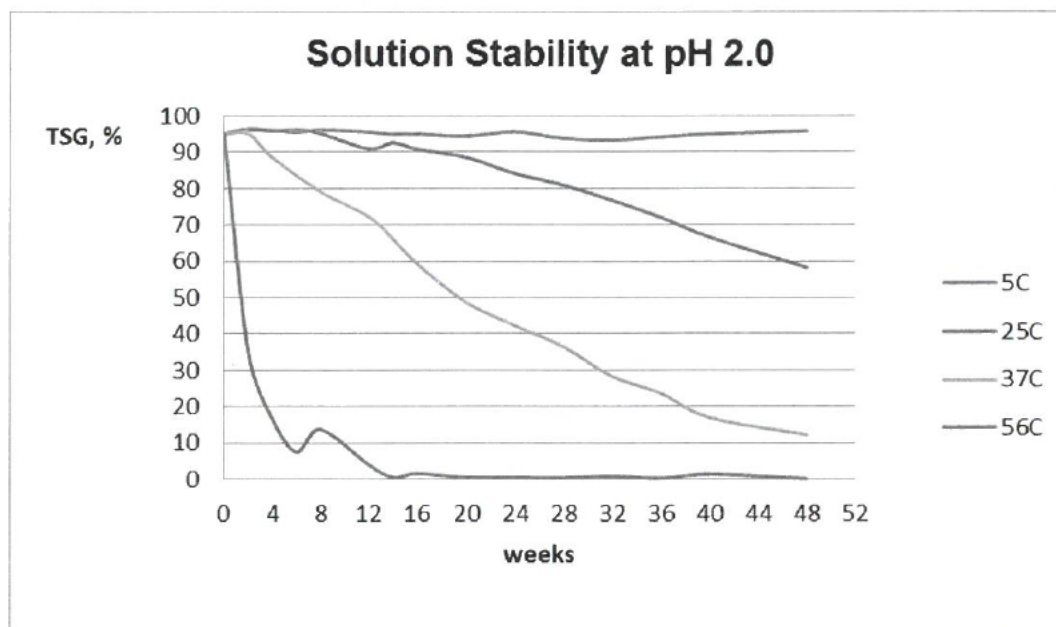
56°C Total Steviol Glycosides, %							
Weeks	pH2	pH3	pH4	pH5	pH6	pH7	pH8
0	95.02	95.21	95.37	95.57	96.12	95.81	95.54
2	35.10	93.15	94.24	94.43	91.99	91.66	93.31
4	16.70	87.00	94.88	93.40	91.08	84.56	89.57
6	7.52	82.40	93.08	92.66	87.85	76.55	86.12
8	13.72	81.84	93.99	93.47	88.38	74.65	84.14
12	4.02	73.34	90.14	89.26	79.87	70.09	78.76
14	0.54	67.52	90.06	88.59	82.23	66.61	77.67
16	1.53	61.27	87.67	85.79	78.43	61.91	75.53
20	0.49	49.36	84.04	82.69	72.32	54.63	65.79
24	0.44	36.60	79.25	77.66	68.83	50.46	61.62
28	0.37	32.11	78.72	76.32	67.53	48.29	62.27
32	0.81	24.06	75.95	71.51	61.16	43.79	53.87
36	0.26	20.74	67.29	68.76	57.57	39.37	50.91
40	1.32	27.58	61.55	62.74	36.88	54.75	49.96
48	0.13	8.37	55.83	60.34	49.80	34.88	41.72



 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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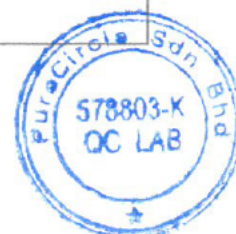
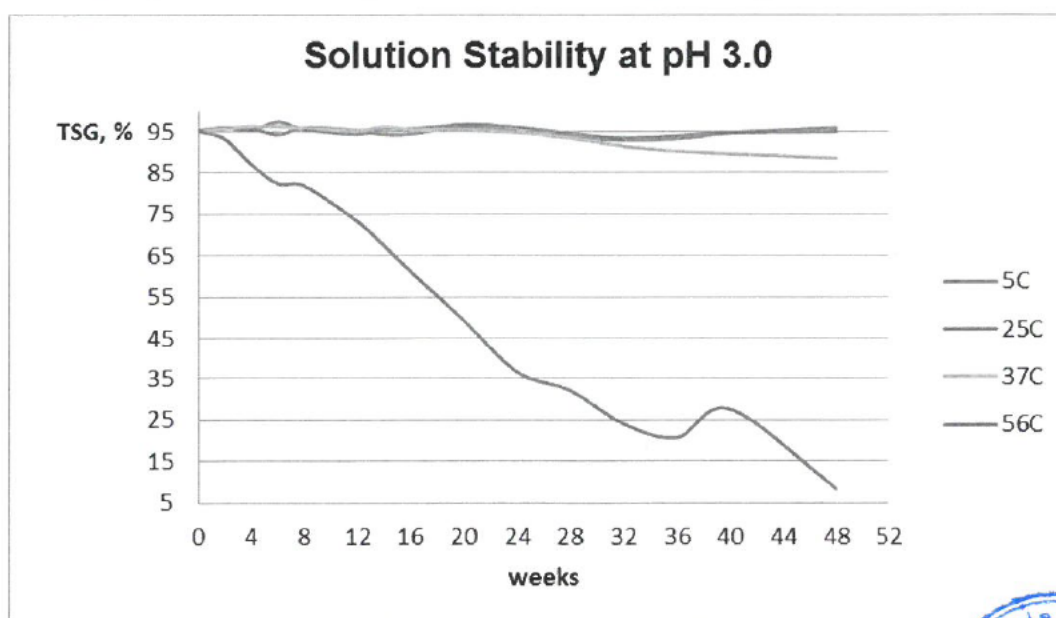
Graph 1


TSG changes of Reb A 50 pH2 solution in different temperatures



Graph 2

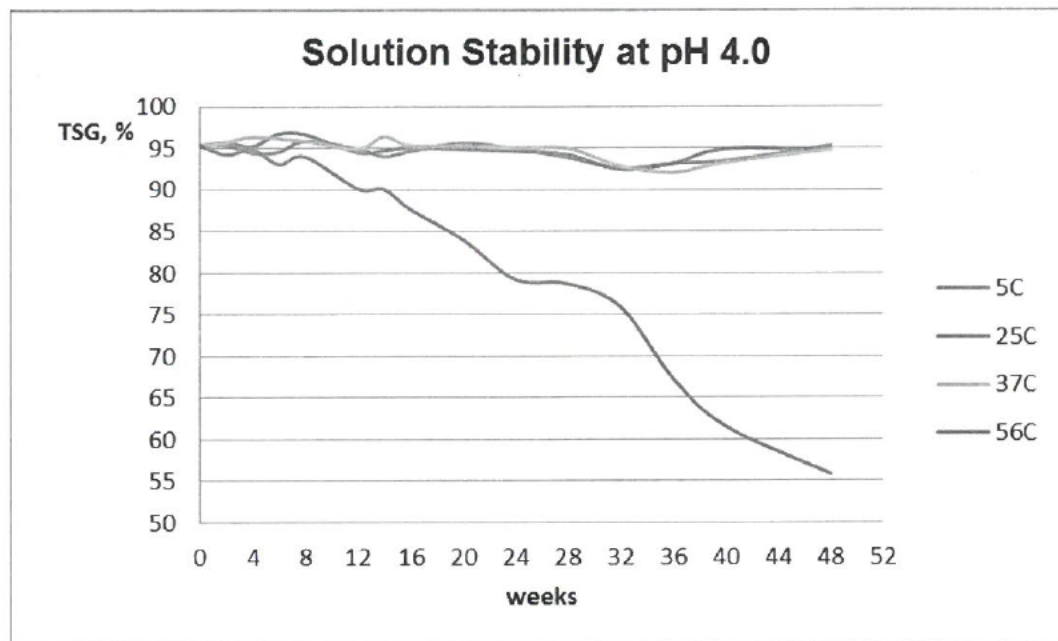
TSG changes of Reb A 50 pH3 solution in different temperatures



 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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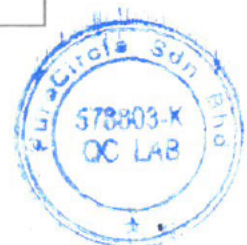
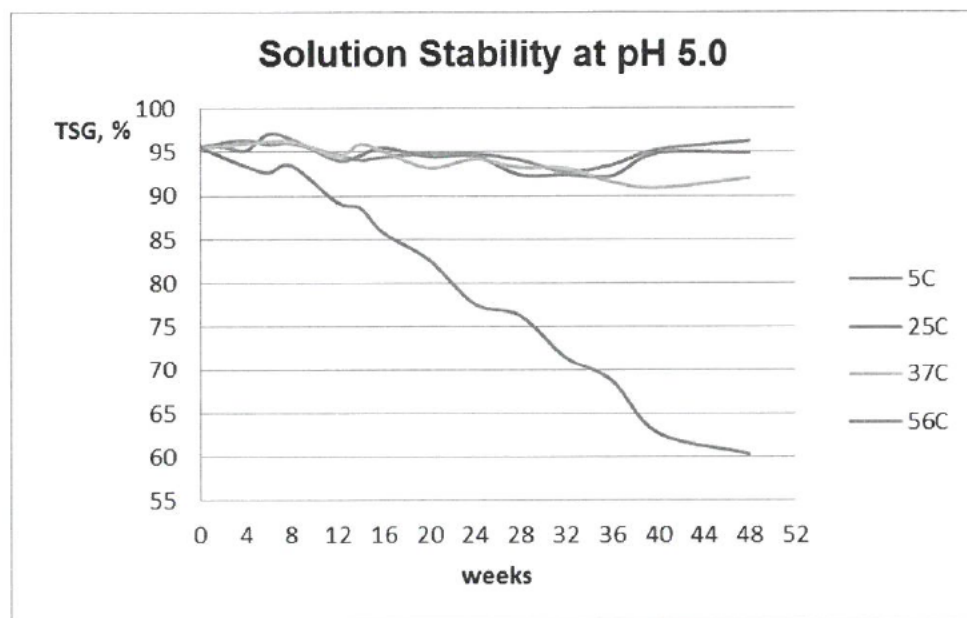
Graph 3


TSG changes of Reb A 50 pH4 solution in different temperatures



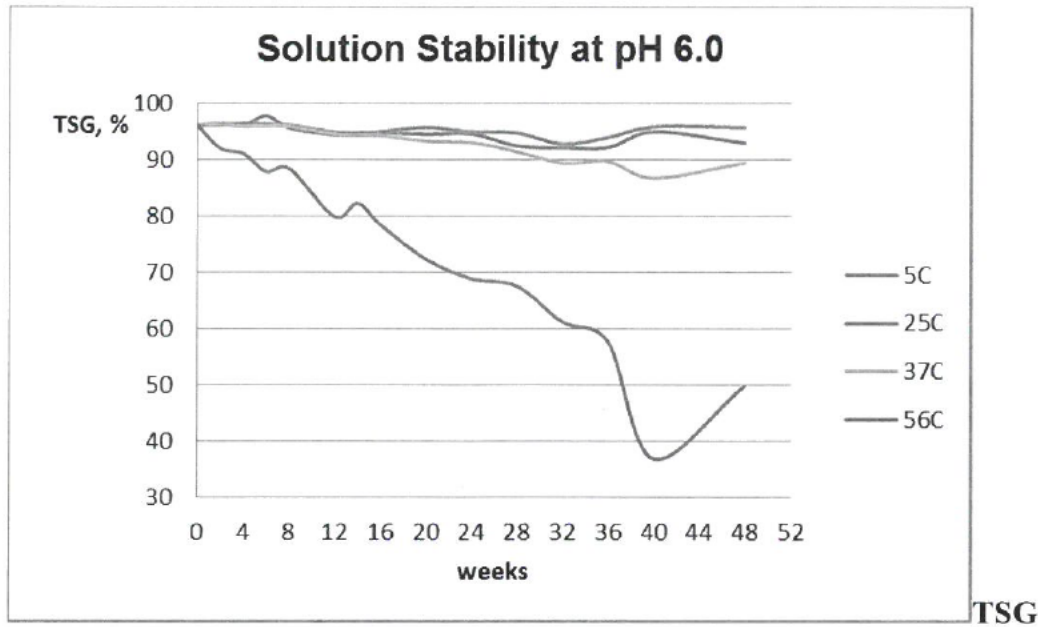
Graph 4

TSG changes of Reb A 50 pH5 solution in different temperatures



 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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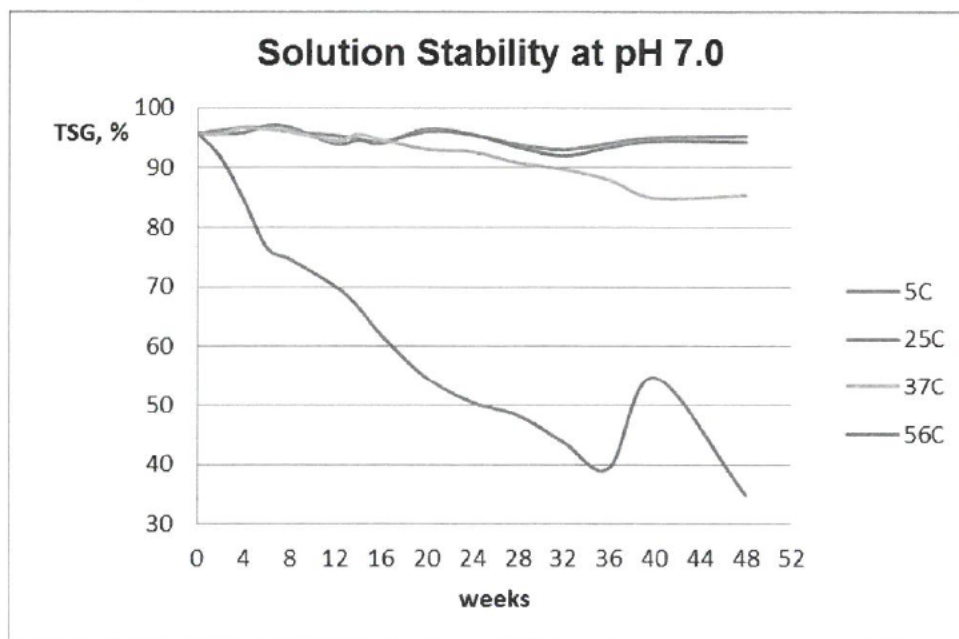
Graph 5




changes of Reb A 50 pH6 solution in different temperatures

Graph 6

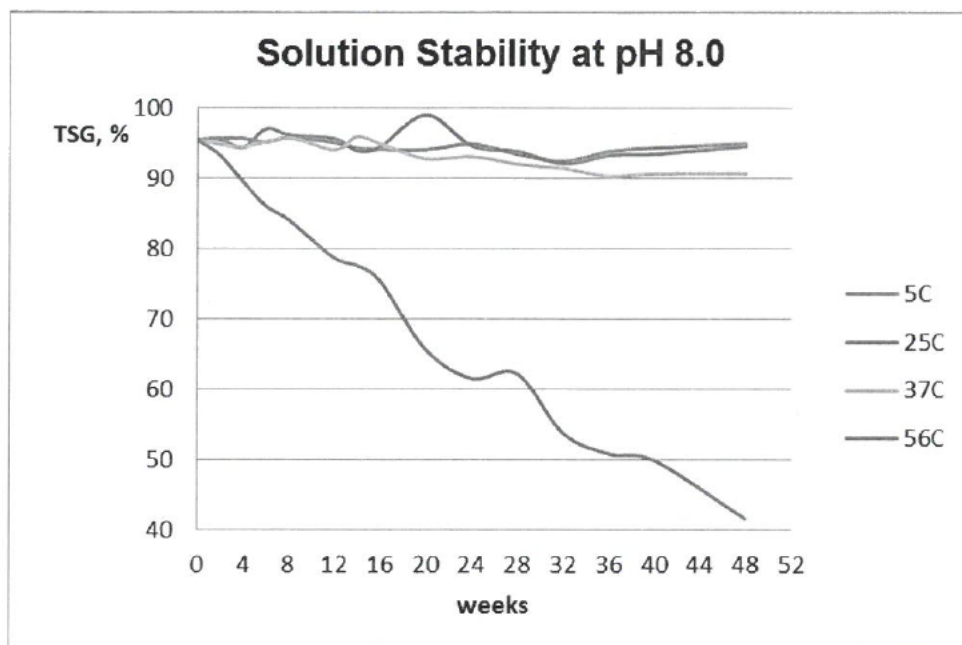
TSG changes of Reb A 50 pH7 solution in different temperatures



 PureCircle Sdn Bhd pH stability of Reb A 50	Report# RebA50_pH_Stability	Issue date: 16/10/15
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Graph 7


TSG changes of Reb A 50 pH8 solution in different temperatures



Reference

1. Steviol Glycosides, FAO JECFA Monographs 10 (2010), P17-21




 PureCircle PureCircle Sdn Bhd	Report# RebA50_Storage_Stability	Issue date: 27/10/15
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Storage stability of Reb A 50 powder		Revision No: 00

Storage Stability of Reb A 50 powder

Prepared by : [Redacted] _____ 27/10/2015
(QC Chemist) **Date**

Approved by : [Redacted] _____ 27/10/15
(QA/QC Manager) **Date**



 PureCircle	PureCircle Sdn Bhd	Report# RebA50_Storage_Stability	Issue date: 27/10/15
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Storage stability of Reb A 50 powder			Revision No: 00

Objective

To determine stability and dynamic changes of Reb A 50 powder produced by PureCircle over 122 weeks of storage at 40°C, 75% RH. The storage stability of Reb A 50 powder is studied by understanding the changes of the Total Steviol Glycosides (TSG) as well as the distribution of different steviol glycosides.

Sample


One sample representing commercial lot of Reb A 50 labeled as “A0912266”.

Standards

1. Rebaudioside A Standard (USP Rockville, MD USA, Lot#F0I077), or equivalent;
2. Stevioside Standard (USP Rockville, MD USA, Lot#F0I080), or equivalent;
3. Rebaudioside B Standard (Chromadex Inc. Irvine, CA USA, Lot#00018237-001), or equivalent.
4. Rebaudioside C Standard (Chromadex Inc. Irvine, CA USA, Lot#00018228-3962), or equivalent;
5. Rebaudioside D Standard (PureCircle Sdn. Bhd., Malaysia, Lot#L03082015), or equivalent;
6. Rebaudioside F Standard (Chromadex Inc. Irvine, CA USA, Lot#00018305-103), or equivalent;
7. Dulcoside A Standard (Chromadex Inc. Irvine, CA USA, Lot#04949-002), or equivalent;
8. Rubusoside Standard, (PureCircle Sdn. Bhd., Malaysia, Lot#270308), or equivalent;
9. Steviolbioside Standard (Chromadex Inc. Irvine, CA USA, Lot#00019349-1821), or equivalent.

Solvents and Reagents



 PureCircle Sdn Bhd Storage stability of Reb A 50 powder	Report# RebA50_Storage_Stability	Issue date: 27/10/15
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1. Acetonitrile, HPLC grade (JT Baker, USA);
2. Water, HPLC grade (JT Baker, USA);
3. Sodium dihydrogen phosphate monohydrate, reagent grade (Merck, Germany);
4. Ortho-phosphoric acid 85%, reagent grade (SYSTEM, USA).

Apparatus


1. Agilent 1200 HPLC system equipped with binary pump (G1312B), auto sampler (G1367D), thermostatted column compartment (G1316B) and DAD detector (G1315C), (Agilent Technologies, USA);
2. Analytical column, Poroshell 120 SB-C18, 4.6x150mm, 2.7 μ m (PN 683975-902), (Agilent Technologies, USA);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Volumetric (class A) and Laboratory glassware.
6. Sonicator

Preparation of Sample

Total 100g of sample was packed in glass containers. The glass container was then placed in the 40°C, 75% humidity chamber. The samples were withdrawn for the HPLC assay in a timely manner.

Samples are prepared by placing 60 \pm 5 mg, recorded to the nearest 0.01 mg, of sample in a 50 mL volumetric flask and diluting to volume with the diluent solution. Sonicate the solution if necessary until dissolved. This will make an approximately 1200 mg/L sample. The sample was prepared duplicated.



 PureCircle Sdn Bhd PureCircle	Report# RebA50_Storage_Stability	Issue date: 27/10/15
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Preparation of Sample

Rebaudioside A and Stevioside standard

Place 40, 50.0, 60, and 70± 5 mg, of the Rebaudioside A standard in four separate 100 ml volumetric flasks while place 20, 30.0, 40, and 50± 5 mg, of the Stevioside standard in four separate 100 mL volumetric flasks. It was then diluted to volume with the diluent to prepare the standards that are approximately 400, 500, 600, and 700 mg/L and 200, 300, 400, and 500 mg/L respectively. The moisture content was measured by Karl Fischer analysis every time a group of standards are prepared.

Analysis

Standard Curve Acceptance Criteria

The criteria of Standard Curve for rebaudioside A and Stevioside to be acceptable for use in the calibration curve, the standard recoveries must be within 100.0 ± 3% for all rebaudioside A and Stevioside concentration levels. No more than one standard may be omitted at each concentration level. The correlation coefficient for the standard curve is acceptable if it is greater than 0.9990.


Rebaudioside A: Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_A) of rebaudioside A in the *Sample solution* in mg/L. Calculate the content (%) of rebaudioside A in the portion of the sample taken by the formula:

$$C_A / C_{SMP} \times 100 \%$$

C_A = Concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)



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Stevioside : Prepare a standard curve for Stevioside by plotting Stevioside peak areas versus concentrations in mg/L, corrected for purity. From the standard curve, calculate the concentration (C_S) of Stevioside in the *Sample solution* in mg/L. Calculate the content (%) of Stevioside in the portion of the sample taken by the formula:

$$C_S / C_{SMP} \times 100 \%$$

C_S = Concentration of Stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)

Other steviol glycosides: Rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusosodie and steviolbioside use the stevioside standard cuve prepared above to calculated the mg/L stevioside for each. Separately calculate the percents of each analyte (rebaudioside B, rebaudioside F, rebaudioside C, dulcoside A, rubusoside and steviolbioside) in the sample taken using the following formula which takes into account the difference in molecular weights between the analytes and stevioside:


$$(C_S \times f_s) / C_{SMP} \times 100 \%$$

C_S = Concentration of Stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

f_s = ratio of the formula weight of others steviol glycosides to the formula weight of stevioside; 1.00 (rebaudioside B), 1.16 (rebaudioside F), 1.18 (rebaudioside C), 0.98 (dulcoside A), 0.80 (rubusoside), and 0.80 (steviolbioside)

C_{SMP} = Concentration of the sample in the *Sample solution* (mg/L)



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Result

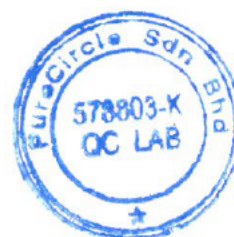
The assay results are summarized in Table 1 and chart 1.

Table 1

Reb A 50 assay of the powder sample kept in amber glass bottle, 40°C, 75% RH

Accelerated study - 40°C and 75%RH

Duration weeks	Lot# A0912266, Steviol Glycosides, % dry basis*									
	<i>Rub</i>	<i>DulA</i>	<i>Stev</i>	<i>RebC</i>	<i>RebF</i>	<i>RebA</i>	<i>RebD</i>	<i>Sbio</i>	<i>RebB</i>	<i>TSG</i>
0	0.3	0.46	18.22	9.58	2.41	64.47	ND	0.3	1.06	96.8
4	0.31	0.48	18	9.61	2.45	64.43	ND	0.33	1.22	96.82
8	0.25	0.39	17.91	9.7	2.06	64.44	ND	0.26	0.97	95.97
12	0.25	0.4	18.03	9.75	2.02	63.28	ND	0.26	1.02	95.01
16	0.26	0.41	18.08	9.77	2.01	63.83	ND	0.27	1.1	95.72
20	0.25	0.41	18.1	9.8	2.01	64.11	ND	0.29	1.11	96.08
24	0.25	0.4	18.12	9.87	2.18	64.44	ND	0.29	1.11	96.66
28	0.26	0.38	17.97	9.7	2.17	64.17	ND	0.26	1.11	96.01
36	0.19	0.35	17.55	9.51	2.53	63.61	ND	0.3	1.14	95.17
44	0.19	0.35	17.55	9.51	2.53	63.61	ND	0.30	1.14	95.17
52	0.20	0.36	17.69	9.60	2.39	63.83	ND	0.39	1.18	95.63
64	0.23	0.37	17.81	9.58	2.11	64.28	ND	0.30	1.18	95.86
72	0.27	0.39	17.75	9.63	2.04	63.84	ND	0.34	1.31	95.58
80	0.25	0.40	17.86	9.65	2.06	63.34	ND	0.36	1.29	95.22
96	0.25	0.35	17.76	9.66	1.93	63.27	ND	0.35	1.24	94.90
106	0.25	0.36	17.56	9.42	1.94	62.49	ND	0.38	1.44	93.84
122	0.26	0.40	17.35	9.38	1.98	61.54	ND	0.37	1.64	92.93




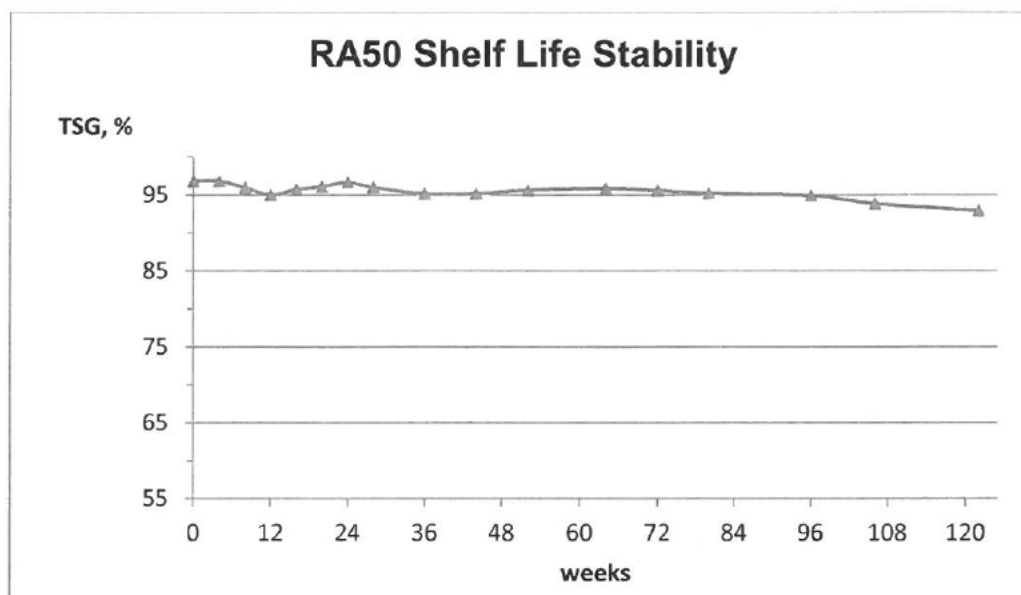
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Chart 1

Dynamic changes of the TSG of Reb A 50



Reference

1. Steviol Glycosides, FAO JECFA Monographs 10 (2010), P17-21

