 <b>PureCircle Sdn Bhd</b> <b>PureCircle</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
		<b>Revision No:</b> 00

## Storage Stability of Rebaudioside X

**Prepared by:**



(Scientist)

19/09/12

Date


**Approved by:**



(Vice President of R&D and Process Development)

19/09/12

Date

 <b>PureCircle</b> <b>PureCircle Sdn Bhd</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
	<b>Revision No:</b> 00	

### **Objective**


To determine storage stability of *Rebaudioside X* produced by *PureCircle*.

### **Samples**

One sample representing commercial lots of *Rebaudioside X* labeled as “PT200212a”.

### **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# 00018227-101), or equivalent;
4. Rebaudioside C standard (Chromadex Inc. Irvine, CA USA, Lot# 00018228-3962), or equivalent;
5. Rebaudioside D standard (Chromadex Inc. Irvine, CA USA, Lot# 00018229-4104), or equivalent;
6. Rebaudioside F standard (Chromadex Inc. Irvine, CA USA, Lot# 00018305-103), or equivalent;
7. Steviolbioside standard (Chromadex Inc. Irvine, CA USA, Lot# 00019349-2871-16), or equivalent;
8. Dulcoside A standard (Chromadex Inc. Irvine, CA USA, Lot# 00004949-002), or equivalent;
9. Rubusoside standard (PureCircle Sdn. Bhd., Malaysia, Lot# 270308), or equivalent;
10. Rebaudioside X standard (PureCircle Sdn. Bhd., Malaysia, Lot# PT031011), or equivalent.

 <b>PureCircle Sdn Bhd</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
		<b>Revision No:</b> 00

### Solvents and Reagents

- Acetonitrile, HPLC grade (JT Baker®, USA);
- Water, HPLC grade (JT Baker®, USA);
- Sodium dihydrogen phosphate monohydrate, NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (Merck®, Germany);
- *ortho*-Phosphoric acid 85% (System®), USA).

### Apparatus

1. Agilent 1100 HPLC system equipped with Quaternary pump (G1311A), auto sampler (G1313A), thermostatted column compartment (G1316A) and MWD detector (G1365B), (Agilent Technologies, USA);
2. Phenomex® Kinetex™ 2.6µm C18 100 Å, LC Column 150 x 4.6 mm (PN 00F-4462-E0);
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
5. Constant climate chamber, KBF 115, (Binder, Germany);
6. Volumetric (class A) and Laboratory glassware.


### Sample Storage

Samples were stored in sealed amber glass vials at

1. 25°C and ambient relative humidity (50-55%);
2. 40°C and 75% relative humidity.

### Sample Solution

Sample solution was prepared at approx. 500 mg/L in *Diluent*. Water content was determined by Karl Fischer method.

 <b>PureCircle Sdn Bhd</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
		<b>Revision No:</b> 00

### Analysis

Analysis and calculations were performed as per JECFA2010 monograph method.  
The assay results are summarized in Table 1-2.

**Table 1**

#### **Rebaudioside X and related steviol glycosides assay**

<b>Duration</b>	<b>Lot# PT200212a in sealed amber glass vial at 25°C, Steviol Glycosides, % dry basis*</b>									
	<i>Reb D</i>	<i>Reb X</i>	<i>Reb A</i>	<i>Stev</i>	<i>Reb F</i>	<i>Reb C</i>	<i>Dul A</i>	<i>Rub</i>	<i>Reb B</i>	<i>Sbio</i>
<b>t=0</b>	1.23	99.93	0.10	ND	ND	ND	ND	ND	0.39	ND
<b>12 weeks</b>	1.20	98.22	0.57	ND	ND	ND	ND	ND	0.52	ND
<b>24 Weeks</b>	1.20	97.31	ND	ND	ND	ND	ND	ND	0.68	0.10
<b>26 weeks</b>	1.21	97.91	ND	ND	ND	ND	ND	ND	0.66	ND

**Table 2**

#### **Rebaudioside X and related steviol glycosides assay**

<b>Duration</b>	<b>Lot# PT200212a in sealed amber glass vial at 40°C and 75% relative humidity, Steviol Glycosides, % dry basis*</b>									
	<i>Reb D</i>	<i>Reb X</i>	<i>Reb A</i>	<i>Stev</i>	<i>Reb F</i>	<i>Reb C</i>	<i>Dul A</i>	<i>Rub</i>	<i>Reb B</i>	<i>Sbio</i>
<b>t=0</b>	1.23	99.93	0.10	ND	ND	ND	ND	ND	0.39	ND
<b>12 weeks</b>	1.17	96.50	0.73	ND	ND	ND	ND	ND	1.10	ND
<b>24 Weeks</b>	1.17	95.53	ND	ND	0.10	ND	ND	ND	1.53	ND
<b>26 weeks</b>	1.20	96.00	ND	ND	ND	ND	ND	ND	1.59	ND

\* Average of 2 duplicates is reported.

RebX, A, B, C, D, F – Rebaudioside X, A, B, C, D, F


Stev – Stevioside

DulA – Dulcoside A

Rub – Rubusoside


Sbio – Steviolbioside

ND – Not detected

<div></div> <div><b>PureCircle</b></div> <div><b>PureCircle Sdn Bhd</b></div> <div><b>Storage Stability of Rebaudioside X</b></div>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
		<b>Revision No:</b> 00


### Reference

1. Stability testing of new drug substances and products, *ICH Q1A(R) stability testing guidelines*, The European Agency for the Evaluation of Medicinal Products, 2000.
2. Steviol glycosides, FAO JECFA monograph 10 (2010), p17-21.

 <b>PureCircle</b> Storage Stability of Rebaudioside X	<b>PureCircle Sdn Bhd</b>	<b>Report #</b> RX_shelf_W26	<b>Issue date:</b> 19/09/2012
			<b>Revision date:</b> --/--/--

## APPENDIX A

### Sample Chromatograms

 <b>PureCircle Sdn Bhd</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
	<b>Revision No:</b> 00	

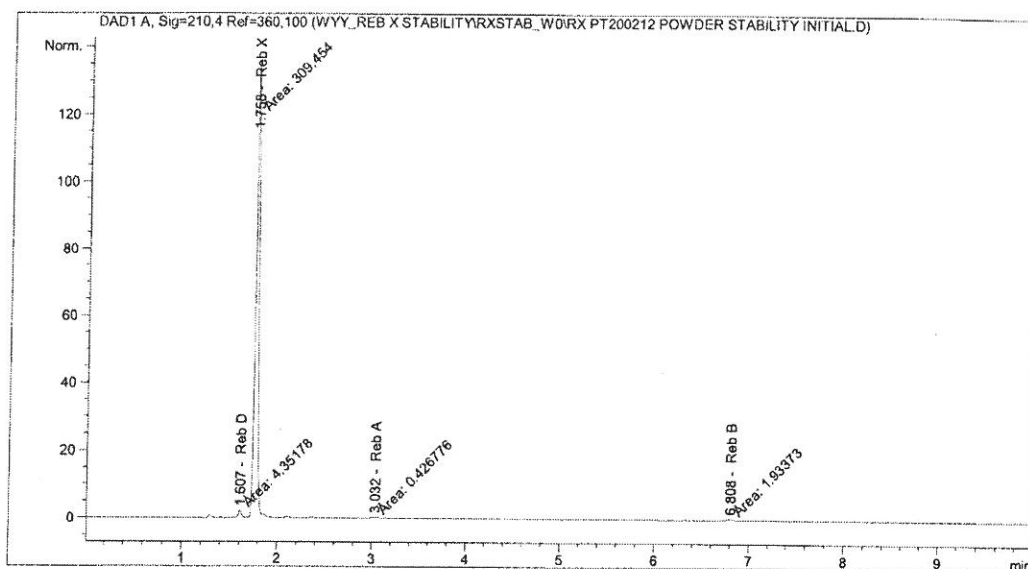
Data File C:\CHEM32\...YY\_REB X STABILITY\RXSTAB\_W0\RX PT200212 POWDER STABILITY INITIAL.D  
Sample Name: RX powder stab

```

=====
Acq. Operator   :                               Seq. Line :   17
Acq. Instrument : Instrument 1                   Location  : Vial 23
Injection Date  : 3/8/2012 7:18:11 PM             Inj       :    1
                                                Inj Volume: 5 µl

Acq. Method     : C:\CHEM32\1\METHODS\C18.M
Last changed    : 3/7/2012 4:22:38 PM
Analysis Method : C:\CHEM32\1\DATA\WYY_REB X STABILITY\RXSTAB_W0\RX_C18_W0.M
Last changed    : 9/14/2012 11:13:42 AM
                  (modified after loading)
Sample Info     : Reb X, Powder stability PT200212 Initial
                  452.1601 mg/L
=====

```



External Standard Report

```

=====
Sorted By      :      Signal
Calib. Data Modified : 9/14/2012 11:13:09 AM
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====


```

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
1.607	MM	4.35178	1.46014	5.56120		Reb D
1.758	MF +	309.45355	1.46014	451.84523		Reb X
3.032	MM	4.26776e-1	1.46014	4.67115e-1		Reb A
3.094		-	-	-		Stev

Instrument 1 9/14/2012 11:16:36 AM

Page 1

 <b>PureCircle Sdn Bhd</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
	<b>Revision No:</b> 00	

Data File C:\CHEM32\...YY\_REB X STABILITY\RXSTAB\_W0\RX PT200212 POWDER STABILITY INITIAL.D  
Sample Name: RX powder stab

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.593		-	-	-		Reb C
3.892		-	-	-		Reb F
4.192		-	-	-		Dul A
5.290		-	-	-		Rub
6.808	MM	1.93373	1.46014	1.76159		Reb B
7.286		-	-	-		SBio


Totals : 459.63514

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

=====  
\*\*\* End of Report \*\*\*



 <b>PureCircle</b> <b>PureCircle Sdn Bhd</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
<b>Storage Stability of Rebaudioside X</b>		<b>Revision No:</b> 00

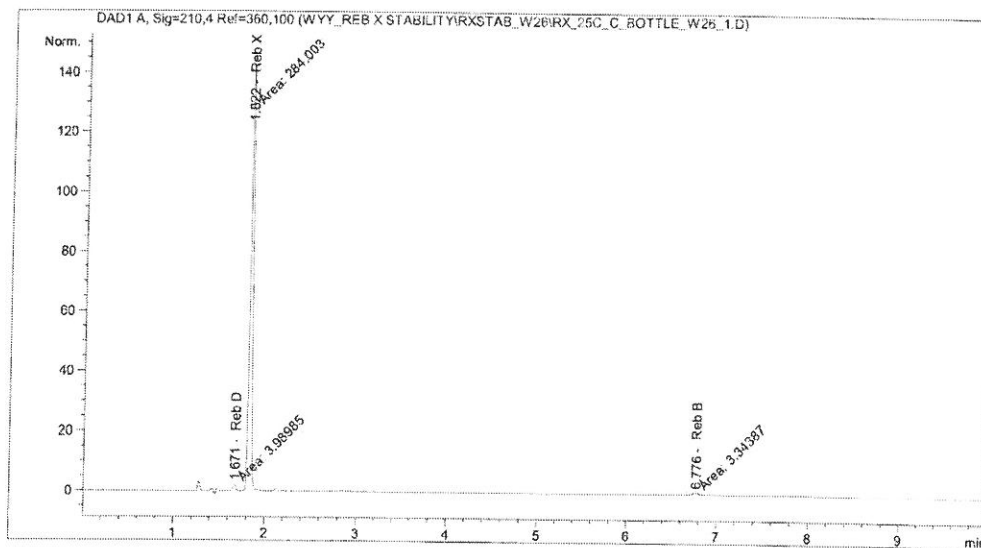
Data File C:\CHEM32\1\DATA\WYY\_REB X STABILITY\RXSTAB\_W26\RX\_25C\_C\_BOTTLE\_W26\_1.D  
Sample Name: RX powstab, 25C, LOT A

```

=====
Acq. Operator   :                               Seq. Line :   16
Acq. Instrument : Instrument 1                  Location  : Vial 53
Injection Date  : 9/6/2012 8:01:16 PM          Inj       :    1
                                           Inj Volume:   5 µl

Acq. Method     : C:\CHEM32\1\METHODS\C18.M
Last changed    : 9/6/2012 3:47:58 PM
Analysis Method : C:\CHEM32\1\DATA\WYY_REB X STABILITY\RXSTAB_W26\RX_C18_W26.M
Last changed    : 9/19/2012 11:24:45 AM
                  (modified after loading)
Sample Info     : Reb X, Powder stability_25C, Vial Lot C:PT200212_ Week
                  25
                  423.2169 mg/L
=====

```



External Standard Report

```

=====
Sorted By       : Signal
Calib. Data Modified : 9/7/2012 9:56:34 AM
Multiplier      : 1.0000
Dilution        : 1.0000
Use Multiplier & Dilution Factor with ISTDs
=====


```

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
1.671	MM	3.98985	1.45791	5.09091		Reb D
1.822	MF +	284.00305	1.45791	414.05201		Reb X
2.979		-	-	-		Reb A

strument 1 9/19/2012 11:25:02 AM

Page 1 of 2

 <b>PureCircle Sdn Bhd</b>	<b>Report #</b> <b>RX_shelf_W26</b>	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
	<b>Storage Stability of Rebaudioside X</b>	<b>Revision No:</b> 00

Data File C:\CHEM32\1\DATA\WYY\_REB X STABILITY\RXSTAB\_W26\RX\_25C\_C\_BOTTLE\_W26\_1.D  
Sample Name: RX powstab, 25C, LOT A


RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.124	-	-	-	-	-	Stev
3.615	-	-	-	-	-	Reb F
3.856	-	-	-	-	-	Reb C
4.165	-	-	-	-	-	Dul A
5.162	-	-	-	-	-	Rub
6.776	MM	3.34387	1.45791	3.04156	-	Reb B
7.243	-	-	-	-	-	SBic

Totals : 422.18448

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

=====  
\*\*\* End of Report \*\*\*

 <b>PureCircle Sdn Bhd</b> <b>Storage Stability of Rebaudioside X</b>	<b>Report #</b> RX_shelf_W26	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
		<b>Revision No:</b> 00

Data File C:\CHEM32\1\DATA\WYY\_REB X STABILITY\RXSTAB\_W26\RX\_HC\_C\_W26\_2.D  
Sample Name: RX powstab, 40C1, LOT A

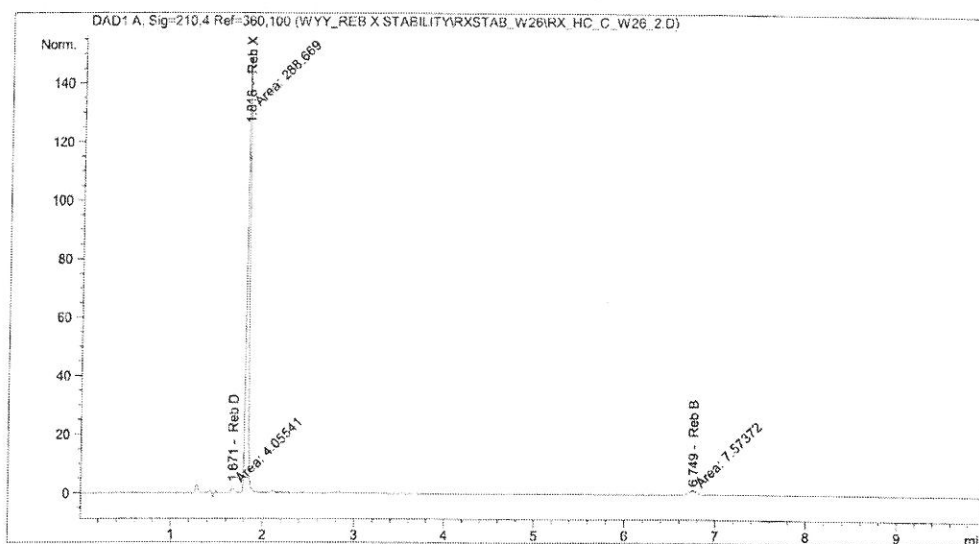
```

=====
Acq. Operator   :                               Seq. Line :   23
Acq. Instrument : Instrument 1                  Location  : Vial 59
Injection Date  : 9/6/2012 10:48:42 PM          Inj       :    2
                                           Inj Volume : 5 µl

Acq. Method     : C:\CHEM32\1\METHODS\C18.M
Last changed    : 9/6/2012 3:47:58 PM
Analysis Method : C:\CHEM32\1\DATA\WYY_REB X STABILITY\RXSTAB_W26\RX_C18_W26.M
Last changed    : 9/19/2012 11:24:45 AM
                  (modified after loading)

Sample Info     : Reb X, humidity chamber, Vial Lot C:PT200212_ Week 26
                  438.9306 mg/L

```



#### External Standard Report

```

Sorted By      : Signal
Calib. Data Modified : 9/7/2012 9:56:34 AM
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs


```

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
1.671	MM	4.05541	1.45791	5.17457		Reb D
1.818	MF +	288.66937	1.45791	420.85510		Reb X
2.973		-	-	-		Reb A
3.118		-	-	-		Stev

Instrument 1 9/19/2012 11:25:56 AM

Page 1 of 2

 <b>PureCircle</b> PureCircle Sdn Bhd	<b>Report #</b> RX_shelf_W26	<b>Issue date:</b> 19/09/2012
		<b>Revision date:</b> --/--/--
	<b>Storage Stability of Rebaudioside X</b>	

Data File C:\CHEM32\1\DATA\WYY\_REB X STABILITY\RXSTAB\_W26\RX\_HC\_C\_W26\_2.D  
Sample Name: RX powstab, 40Cl, LOT A

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.608	-	-	-	-	-	Reb F
3.848	-	-	-	-	-	Reb C
4.157	-	-	-	-	-	Dul A
5.152	-	-	-	-	-	Rub
6.749	MM	7.57372	1.45791	6.88900	-	Reb B
7.228	-	-	-	-	-	SBio

Totals : 432.91867

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\* End of Report \*\*\*