
 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00





**Thermal Stability of Rebaudioside M 95% and
Rebaudioside M 50%
14th September 2014**

Prepared by: _____ **Date** _____


(Scientist)

Approved by: _____ **Date** _____


(Vice President, R&D and Process Development)

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

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Objective


To determine the thermal stability of Rebaudioside M 95% and Rebaudioside M 50% produced by *PureCircle*.

Samples

Two samples representing commercial lots of *Rebaudioside M 95%* labelled as PT130814 and *Rebaudioside M 50%* labeled as PT240714.

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;

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

10. Rebaudioside M standard (Chromadex Inc. Irvine, CA USA, Lot# 00018234-133), or equivalent.

Solvents and Reagents


- Acetonitrile (ACN), HPLC grade (JT Baker[®]);
- Water, HPLC grade (JT Baker[®], USA);
- Sodium dihydrogen phosphate monohydrate, NaH₂PO₄·H₂O (Merck[®]);
- Disodium hydrogen orthophosphate anhydrous, Na₂HPO₄ (Fischer Scientific[®]);
- *ortho*-Phosphoric acid 85% (System[®]);

Apparatus

- 1) *Agilent[®] 1100* HPLC system equipped with quaternary pump (G1311A), auto sampler (G1313A), thermostated column compartment (G1316A), MWD UV Detector (G1365B) and ChemStation (Rev. A.10.02) software (Agilent Technologies, USA);
- 2) *Agilent[®] Poroshell 120 SB-C18* analytical column; 4.6x150mm, 2.7µm, 120Å (PN 683975-902);
- 3) Analytical balance, XS205, (Mettler Toledo, USA);
- 4) *Heidolph[®]* oil bath;
- 5) Volumetric (class A) and laboratory glassware.

Sample solution

Each sample solution was prepared in 0.1M sodium phosphate buffer, pH 6.5.

 PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Sample solution treatment

Sample solutions was incubated at 100°C for 10 minutes.

Analysis

Analysis of solutions before and after incubation and calculations were performed as per JECFA2010 monograph method.

The assay results are summarized in Table 1 and Table 2.

Table 1

Rebaudioside M 95% assay

Sample ID	Steviol Glycosides, mg/L										TSG, mg/L
	<i>Reb D</i>	<i>Reb M</i>	<i>Reb A</i>	<i>Stev</i>	<i>Reb F</i>	<i>Reb C</i>	<i>DulA</i>	<i>Rub</i>	<i>Reb B</i>	<i>Sbio</i>	
Initial	10.99	403.12	2.22	<1	<1	<1	<1	<1	1.10	<1	417.43
100 C , 10 min	10.89	407.44	2.33	<1	<1	<1	<1	<1	0.95	<1	421.61

Table 2

Rebaudioside M 50% assay

Sample ID	Steviol Glycosides, mg/L										TSG, mg/L
	<i>Reb D</i>	<i>Reb M</i>	<i>Reb A</i>	<i>Stev</i>	<i>Reb F</i>	<i>Reb C</i>	<i>DulA</i>	<i>Rub</i>	<i>Reb B</i>	<i>Sbio</i>	
Initial	174.74	238.64	29.82	<1	<1	<1	<1	<1	1.27	<1	444.47
100 C , 10 min	176.78	240.08	30.68	<1	<1	<1	<1	<1	1.37	<1	448.92

Reb M, A, B, C, D, F – Rebaudioside M, A, B, C, D, F

Stev – Stevioside

DulA – Dulcoside A


Rub – Rubusoside

Sbio – Steviolbioside

TSG – Total Steviol Glycosides content

Reference


1. Steviol glycosides, FAO JECFA monograph 10 (2010), p17-21.

 PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

APPENDIX A

Sample Chromatograms

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM96_INITIAL.D

Sample Name: Reb M 96

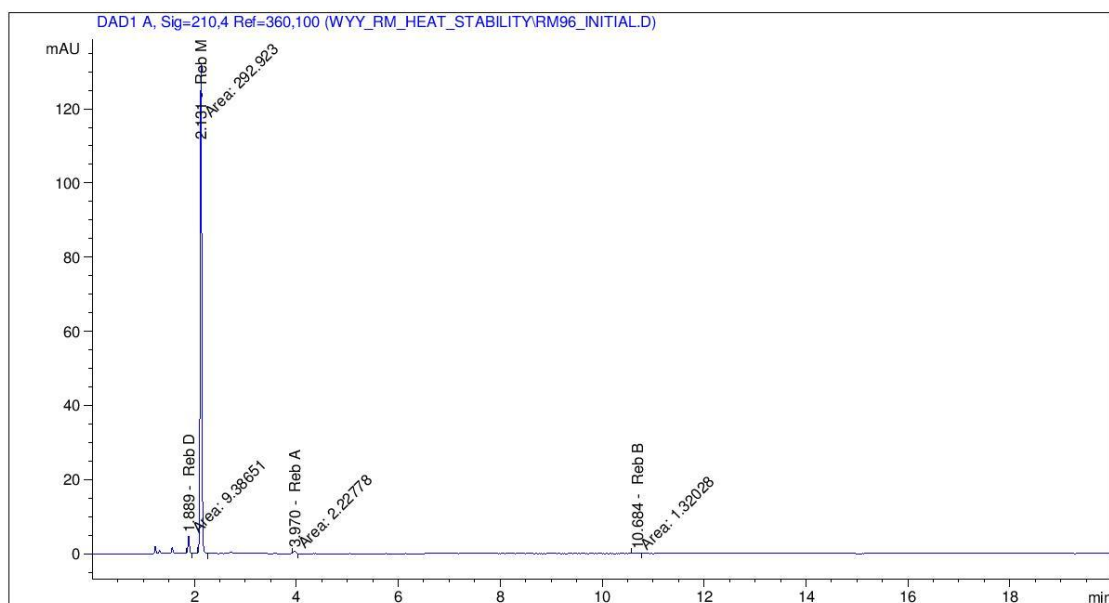
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=====
Acq. Operator   :                               Seq. Line :    2
Acq. Instrument : HPLC 8                       Location  : Vial 1
Injection Date  : 9/14/2014 10:40:06 AM         Inj       :    1
                                                Inj Volume: 5 µl

Acq. Method     : C:\CHEM32\1\METHODS\WYY_C18.M
Last changed    : 9/14/2014 10:18:21 AM
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\WYY_RM_HEAT.M
Last changed    : 9/14/2014 3:34:38 PM
                  (modified after loading)
Method Info     : C18 column

Sample Info     : Reb M 96%, initial
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External Standard Report


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Sorted By      :      Signal
Calib. Data Modified :      Sunday, September 14, 2014 12:24:56 PM
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
1.889	MF	9.38651	1.17057	10.98760		Reb D
2.131	FM	292.92261	1.37620	403.12094		Reb M

 PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM96_INITIAL.D

Sample Name: Reb M 96

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.970	MM +	2.22778	9.98739e-1	2.22497		Reb A
4.169		-	-	-		Stev
5.030		-	-	-		Reb F
5.508		-	-	-		Reb C
5.936		-	-	-		Dul A
7.648		-	-	-		Rub
10.684	MM	1.32028	9.98739e-1	1.09709		Reb B
11.248		-	-	-		Sbio


Totals : 417.43060

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

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

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM96_HEAT.D

Sample Name: Reb M 96

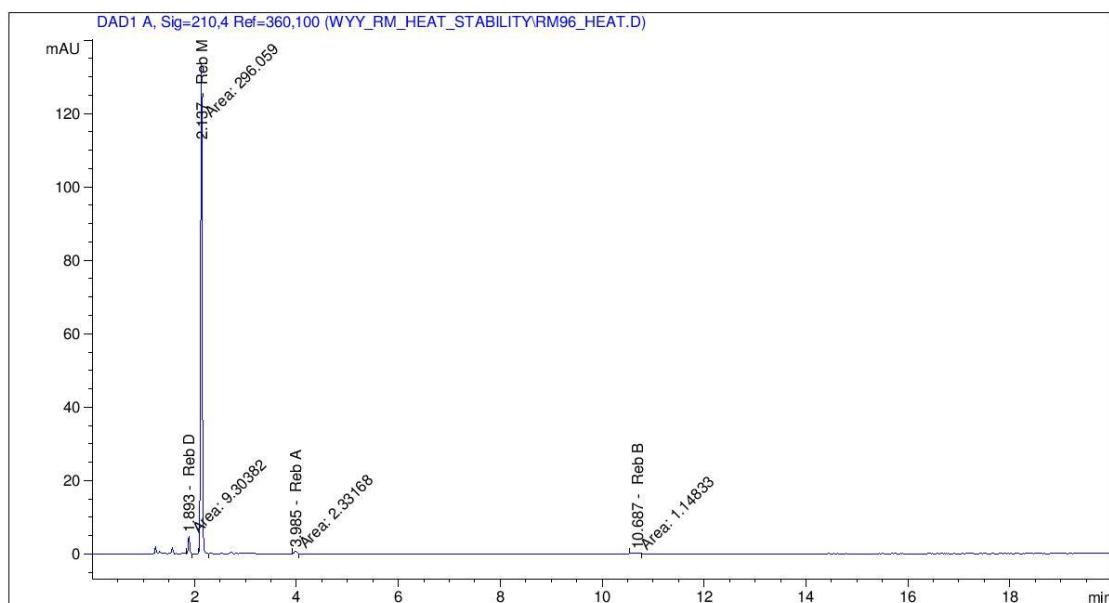
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=====
Acq. Operator   :                               Seq. Line :    3
Acq. Instrument : HPLC 8                       Location  : Vial 2
Injection Date  : 9/14/2014 11:01:56 AM         Inj       :    1
                                                Inj Volume: 5 µl

Acq. Method     : C:\CHEM32\1\METHODS\WYY_C18.M
Last changed    : 9/14/2014 10:18:21 AM
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\WYY_RM_HEAT.M
Last changed    : 9/14/2014 3:32:35 PM
                  (modified after loading)
Method Info     : C18 column

Sample Info     : Reb M 96%, heat at 100C, 10min
=====

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External Standard Report


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=====
Sorted By      :      Signal
Calib. Data Modified :      Sunday, September 14, 2014 12:24:56 PM
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
1.893	MF	9.30382	1.17057	10.89080		Reb D
2.137	MF	296.05881	1.37620	407.43699		Reb M

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM96_HEAT.D

Sample Name: Reb M 96

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.985	MM +	2.33168	9.98739e-1	2.32874		Reb A
4.185		-	-	-		Stev
5.049		-	-	-		Reb F
5.530		-	-	-		Reb C
5.959		-	-	-		Dul A
7.677		-	-	-		Rub
10.687	MM	1.14833	9.98739e-1	9.54205e-1		Reb B
11.292		-	-	-		Sbio


Totals : 421.61074

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

=====
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 PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM50_INITIAL.D

Sample Name: Reb M 50

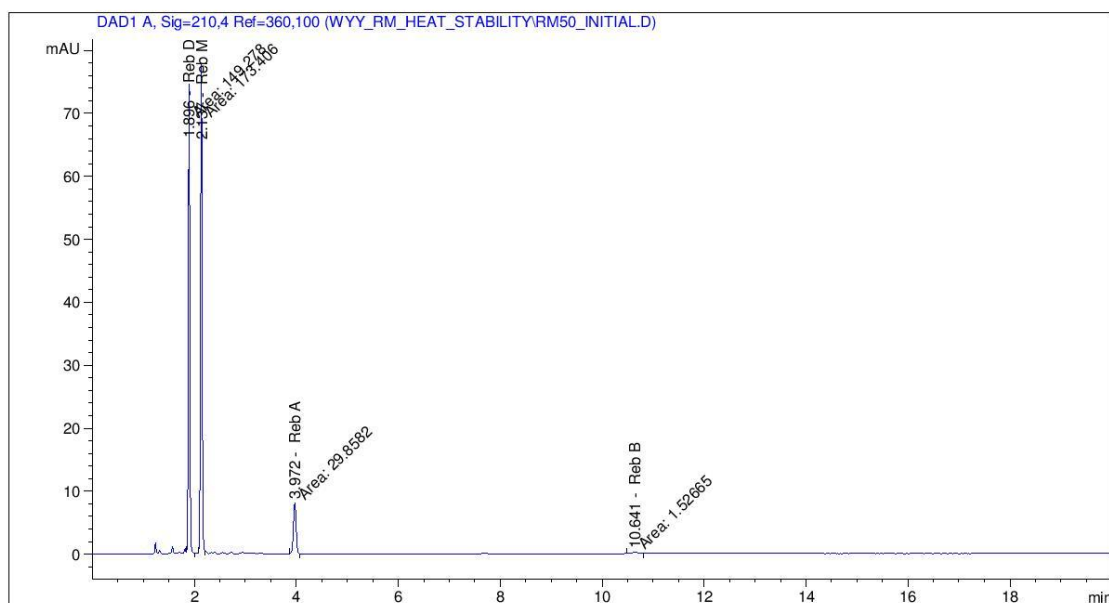
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=====
Acq. Operator   :                               Seq. Line :    4
Acq. Instrument : HPLC 8                       Location  : Vial 3
Injection Date  : 9/14/2014 11:23:47 AM         Inj       :    1
                                                Inj Volume: 5 µl

Acq. Method     : C:\CHEM32\1\METHODS\WYY_C18.M
Last changed    : 9/14/2014 10:18:21 AM
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\WYY_RM_HEAT.M
Last changed    : 9/14/2014 3:29:09 PM
                  (modified after loading)
Method Info     : C18 column

Sample Info     : Reb M 50%, initial
=====

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External Standard Report


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Sorted By      : Signal
Calib. Data Modified : Sunday, September 14, 2014 12:24:56 PM
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
1.896	MF	149.27811	1.17057	174.74089		Reb D
2.137	MF	173.40623	1.37620	238.64216		Reb M

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM50_INITIAL.D

Sample Name: Reb M 50

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.972	MM +	29.85821	9.98739e-1	29.82057		Reb A
4.171		-	-	-		Stev
5.032		-	-	-		Reb F
5.511		-	-	-		Reb C
5.939		-	-	-		Dul A
7.651		-	-	-		Rub
10.641	MM	1.52665	9.98739e-1	1.26857		Reb B
11.253		-	-	-		Sbio


Totals : 444.47219

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

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 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM50_HEAT.D

Sample Name: Reb M 50

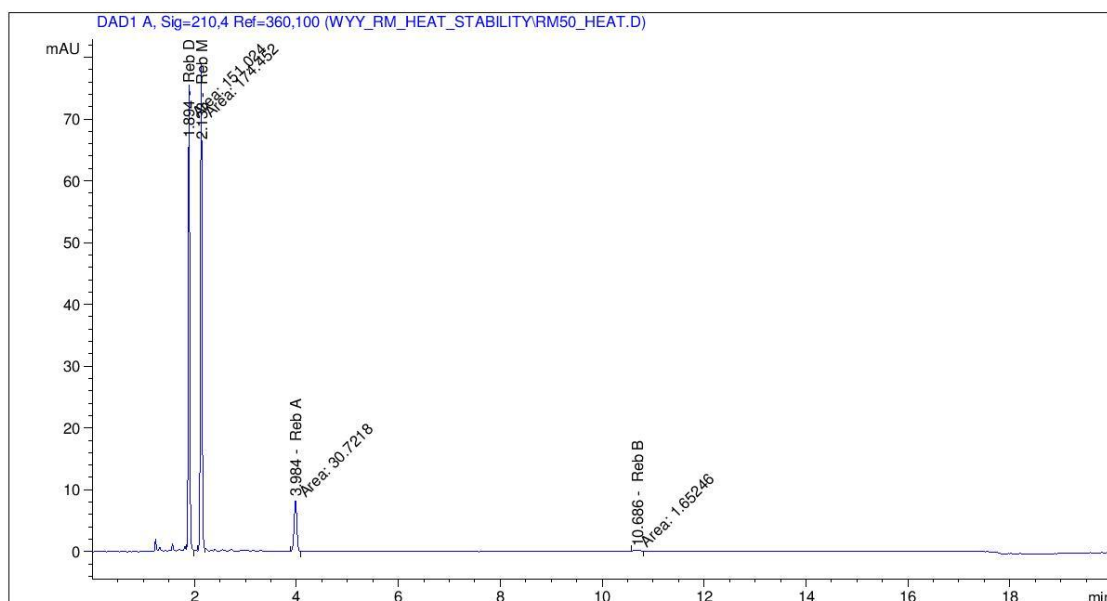
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=====
Acq. Operator   :                               Seq. Line :    5
Acq. Instrument : HPLC 8                       Location  : Vial 4
Injection Date  : 9/14/2014 11:45:40 AM         Inj       :    1
                                                Inj Volume: 5 µl

Acq. Method     : C:\CHEM32\1\METHODS\WYY_C18.M
Last changed    : 9/14/2014 10:18:21 AM
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\WYY_RM_HEAT.M
Last changed    : 9/14/2014 3:27:47 PM
                  (modified after loading)
Method Info     : C18 column

Sample Info     : Reb M 50%, heat at 100C, 10min
=====

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External Standard Report


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=====
Sorted By      :      Signal
Calib. Data Modified :      Sunday, September 14, 2014 12:24:56 PM
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
1.894	MF	151.02409	1.17057	176.78469		Reb D
2.133	MF	174.45154	1.37620	240.08071		Reb M

 PureCircle PureCircle Sdn Bhd	Report#: WYY_Thermal Stability_RM	Issue date: 14/09/14
		Revision date: -----
Thermal Stability of Rebaudioside M 95% and Rebaudioside M 50%		Revision No: 00

CONFIDENTIAL

Data File C:\CHEM32\1\DATA\WYY_RM_HEAT_STABILITY\RM50_HEAT.D

Sample Name: Reb M 50

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
3.984	MM +	30.72184	9.98739e-1	30.68311		Reb A
4.183		-	-	-		Stev
5.047		-	-	-		Reb F
5.528		-	-	-		Reb C
5.957		-	-	-		Dul A
7.675		-	-	-		Rub
10.686	MM	1.65246	9.98739e-1	1.37312		Reb B
11.288		-	-	-		Sbio

Totals : 448.92163

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

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