Appendix 1.2 Stevia Extract (≥90% Total Steviol Glycosides) Certificate of Analysis

Shandong Shengxiangyuan Biotechnology Co.,Ltd
山东圣香远生物科技有限公司
Adress:East of chuangye road,south of north fangzhi road,.qufu,China,
Tel:0086-537-4483369 Fax:0086-537-4400999

Certificate Of Analysis

Product Name: Organic Rebaudioside A 50% (SG90%) Manufacture Date: 2016.06.18
Latin Name: Stevia Rebaudiana Bertoni Expire Date: 2018.06.17
Batch No: 20160618 Batch Quantity:1000kgs

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance Odor	White fine powder Characteristic	White fine powder Characteristic	Visual Gustation
CHEMICAL TESTS			
Total Steviol Glucosides(% dry basis)	≥90	90.23	HPLC
Rebaudioside A %	≥50	51.21	HPLC
Loss on Drying (%)	≤4.00	3.28	CP/USP
Ash (%)	≤0.20	0.17	GB(1g/580C/2hrs
Sweetness times	≥320	≥320	
PH (1% solution)	4.5-7.0	5.0	
Specific Optical Rotation	-30°~-38°	-33°	GB
Specific Absorbance	≤0.05	0.03	GB
Lead (ppm)	≤0.1	0.053	CP
Arsenic(ppm)	≤0.1	Negative	CP
Cadmium (ppm)	≤0.1	Negative	CP
Mercury (ppm)	≤0.1	0.037	CP
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
E.Coli(cfu/g)	Negative	Negative	CP/USP
Yeast&Mold(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP

Storage: in cool and dry place,keep away from strong light and heat
Package: 25kg drum or carton (two food grade bags inside)
Country of Original : China
Note:NON-GMO NON-ALLERGEN

INSPECTION:Lin Li **RECHECK: Na Chen**

Appendix 2 Certificates of Analysis for Multiple Batches of SXY Stevia® Total Steviol Glycosides 95% Purified Steviol Glycosides

Appendix 2.1 SXY Stevia® Total Steviol Glycosides 95% Batch (b) (6)

Appendix 2.2 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 2.3 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 2.4 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 2.5 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 2.1 SXY Stevia® Total Steviol Glycosides 95% Batch 20160913

Shandong Shengxiangyuan Biotechnology Co.,Ltd
山东圣香远生物科技有限公司
Adress: No.9 East Haiquan Rd., Qufu,Jining,Shandong Province China,
Tel:0086-537-4482369 Fax:0086-537-4400999

Certificate Of Analysis

Product Name: total steviol glycosides 95% Manufacture Date: 2016.09.13
Latin Name: Stevia Rebaudiana Bertoni Expire Date: 2018.09.12
Plant part used: stevia leaves Batch No: 20160913 Batch Quantity: 1000kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance Odor	White fine powder Characteristic	White fine powder Characteristic	Visual Gustation
CHEMICAL TESTS			
Steviol glycosides (% dry basis)	≥95	95.52	HPLC
Rebaudioside A %	50-70	61.18	HPLC
Rebaudioside B%	1.0-3.0	1.92	HPLC
Rebaudioside C %	2.0-5.0	2.44	HPLC
Rebaudioside D %	0.3-1.5	0.47	HPLC
Rebaudioside F %	0.5-1.5	0.66	HPLC
Stevioside %	20.0-30.0	25.33	HPLC
DulcosideA %	0.3-1.0	0.36	HPLC
Rubusodide %	2.0-4.0	2.42	HPLC
Steviobioside %	0-1.0	0.74	HPLC
Sweetness times	280-320	300	
Loss on Drying (%)	≤4.00	3.10	CP/USP
Ash (%)	≤0.1	0.09	GB(1g/580C/2hrs)
PH (1% solution)	4.5-6.0	5.20	
Particle Size		100% pass 80	
Specific Optical Rotation	-30°~-38°	-34.5°	GB
Specific Absorbance	≤0.05	0.025	GB
Lead (ppm)	≤0.1	0.05	CP
Arsenic(ppm)	≤0.1	Below detection limit	CP
Cadmium (ppm)	≤0.1	Below detection limit	CP
Mercury (ppm)	≤0.1	0.05	CP
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Yeast&Mold(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP

Solvents

Methanol (ppm)	≤200	76	CP/USP
Ethanol (ppm)	≤5000	583	CP/USP

Package:20kg drum or carton (two l food grade bags inside)
Country of Original: China
Note:NON-GMO NON-ALLERGEN

INSPECTION:Jin Meng Xu **RECHECK: Bao Juan Peng**

Appendix 2.2 SXY Stevia® Total Steviol Glycosides 95% Batch 20160916

Shandong Shengxiangyuan Biotechnology Co.,Ltd
山东圣香远生物科技有限公司
Adress: No.9 East Haiguan Rd., Qufu,Jining,Shandong Province China,
Tel:0086-537-4482369 Fax:0086-537-4400999

Certificate Of Analysis

Product Name: total steviol glycosides 95% Manufacture Date: 2016.09.16
Latin Name: Stevia Rebaudiana Bertoni Expire Date: 2018.09.15
Plant part used: stevia leaves Batch No: 20160916 Batch Quantity: 1000kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance Odor	White fine powder Characteristic	White fine powder Characteristic	Visual Gustation
CHEMICAL TESTS			
Steviol glycosides (% dry basis)	≥95	95.32	HPLC
Rebaudioside A %	50-70	61.16	HPLC
Rebaudioside B%	1.0-3.0	1.66	HPLC
Rebaudioside C %	2.0-5.0	1.87	HPLC
Rebaudioside D %	0.3-1.5	0.34	HPLC
Rebaudioside F %	0.5-1.5	0.59	HPLC
Stevioside %	20.0-30.0	25.40	HPLC
DulcosideA %	0.3-1.0	0.33	HPLC
Rubusodide %	2.0-4.0	3.07	HPLC
Steviobioside %	0-1.0	0.91	HPLC
Sweetness times	280-320	300	
Loss on Drying (%)	≤4.00	3.26	CP/USP
Ash (%)	≤0.1	0.08	GB(1g/580C/2hrs)
PH (1% solution)	4.5-6.0	5.20	
Particle Size		100% pass 80	
Specific Optical Rotation	-30°~-38°	-34.5°	GB
Specific Absorbance	≤0.05	0.027	GB
Lead (ppm)	≤0.1	0.05	CP
Arsenic(ppm)	≤0.1	Below detection limit	CP
Cadmium (ppm)	≤0.1	Below detection limit	CP
Mercury (ppm)	≤0.1	0.05	CP
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Yeast&Mold(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP

Solvents

Methanol (ppm)	≤200	89	CP/USP
Ethanol (ppm)	≤5000	397	CP/USP

Package:20kg drum or carton (two l food grade bags inside)
Country of Original: China **Note:NON-GMO NON-ALLERGEN**

INSPECTION:Jin Meng Xu **RECHECK: Bao Juan Peng**

Appendix 2.3 SXY Stevia® Total Steviol Glycosides 95% Batch 20160914

Shandong Shengxiangyuan Biotechnology Co.,Ltd
山东圣香远生物科技有限公司
Adress: No.9 East Haiguan Rd., Qufu Jining Shandong Province China,
Tel:0086-537-4482369 Fax:0086-537-4400999

Certificate Of Analysis

Product Name: total steviol glycosides 95% Manufacture Date: 2016.09.14
Latin Name: Stevia Rebaudiana Bertoni Expire Date: 2018.09.13
Plant part used: stevia leaves Batch No: 20160914 Batch Quantity: 1000kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance Odor	White fine powder Characteristic	White fine powder Characteristic	Visual Gustation
CHEMICAL TESTS			
Steviol glycosides (% dry basis)	≥95	95.57	HPLC
Rebaudioside A %	50-70	63.60	HPLC
Rebaudioside B%	1.0-3.0	1.76	HPLC
Rebaudioside C %	1.0-5.0	1.89	HPLC
Rebaudioside D %	0.3-1.5	0.70	HPLC
Rebaudioside F %	0.5-1.5	0.57	HPLC
Stevioside %	20.0-30.0	23.15	HPLC
DulcosideA %	0.3-1.0	0.40	HPLC
Rubusodide %	2.0-4.0	2.77	HPLC
Steviobioside %	0-1.0	0.73	HPLC
Loss on Drying (%)	≤4.00	3.07	CP/USP
Sweetness times	280-320	300	
Ash (%)	≤0.1	0.09	GB(1g/580C/2hrs)
PH (1% solution)	4.5-6.0	5.20	
Particle Size		100% pass 80	
Specific Optical Rotation	-30°~-38°	-34.5°	GB
Specific Absorbance	≤0.05	0.030	GB
Lead (ppm)	≤0.1	0.05	CP
Arsenic(ppm)	≤0.1	Below detection limit	CP
Cadmium (ppm)	≤0.1	Below detection limit	CP
Mercury (ppm)	≤0.1	0.05	CP
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Yeast&Mold(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP

Solvents

Methanol (ppm)	≤200	94	CP/USP
Ethanol (ppm)	≤5000	502	CP/USP

Package:20kg drum or carton (two l food grade bags inside)
Country of Original : China **Note:NON-GMO NON-ALLERGEN**

INSPECTION:Jin Meng Xu **RECHECK: Bao Juan Peng**

Appendix 2.4 SXY Stevia® Total Steviol Glycosides 95% Batch 20160917

Shandong Shengxiangyuan Biotechnology Co.,Ltd
山东圣香远生物科技有限公司
Adress: No.9 East Haiguan Rd., Qufu,Jining,Shandong Province China,
Tel:0086-537-4482369 Fax:0086-537-4400999

Certificate Of Analysis

Product Name: total steviol glycosides 95% Manufacture Date: 2016.09.17
Latin Name: Stevia Rebaudiana Bertoni Expire Date: 2018.09.16
Plant part used: stevia leaves Batch No: 20160917 Batch Quantity: 1000kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance Odor	White fine powder Characteristic	White fine powder Characteristic	Visual Gustation
CHEMICAL TESTS			
Steviol glycosides (% dry basis)	≥95	95.70	HPLC
Rebaudioside A %	50-70	62.58	HPLC
Rebaudioside B%	1.0-3.0	1.74	HPLC
Rebaudioside C %	1.5-5.0	1.82	HPLC
Rebaudioside D %	0.3-1.5	0.69	HPLC
Rebaudioside F %	0.5-1.5	0.56	HPLC
Stevioside %	20.0-30.0	24.10	HPLC
DulcosideA %	0.3-1.0	0.37	HPLC
Rubusodide %	2.0-4.0	2.89	HPLC
Steviobioside %	0-1.0	0.94	HPLC
Sweetness times	280-320	300	
Loss on Drying (%)	≤4.00	3.32	CP/USP
Ash (%)	≤0.1	0.09	GB(1g/580C/2hrs)
PH (1% solution)	4.5-6.0	5.20	
Particle Size		100% pass 80	
Specific Optical Rotation	-30°~-38°	-34.5°	GB
Specific Absorbance	≤0.05	0.031	GB
Lead (ppm)	≤0.1	0.05	CP
Arsenic(ppm)	≤0.1	Below detection limit	CP
Cadmium (ppm)	≤0.1	Below detection limit	CP
Mercury (ppm)	≤0.1	0.05	CP
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Yeast&Mold(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP

Solvents

Methanol (ppm)	≤200	99	CP/USP
Ethanol (ppm)	≤5000	503	CP/USP

Package:20kg drum or carton (two l food grade bags inside)
Country of Original: China
Note:NON-GMO NON-ALLERGEN

INSPECTION:Jin Meng Xu **RECHECK: Bao Juan Peng**

Appendix 2.5 SXY Stevia® Total Steviol Glycosides 95% Batch 20160918

Shandong Shengxiangyuan Biotechnology Co.,Ltd
山东圣香远生物科技有限公司
Adress: No.9 East Haiquan Rd., Qufu Jining Shandong Province China,
Tel:0086-537-4482369 Fax:0086-537-4400999

Certificate Of Analysis

Product Name: total steviol glycosides 95% Manufacture Date: 2016.09.18
Latin Name: Stevia Rebaudiana Bertoni Expire Date: 2018.09.17
Plant part used: stevia leaves Batch No: 20160918 Batch Quantity: 1000kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance Odor	White fine powder Characteristic	White fine powder Characteristic	Visual Gustation
CHEMICAL TESTS			
Steviol glycosides (% dry basis)	≥95	95.88	HPLC
Rebaudioside A %	50-70	62.63	HPLC
Rebaudioside B%	1.0-3.0	1.82	HPLC
Rebaudioside C %	1.5-5.0	2.01	HPLC
Rebaudioside D %	0.3-1.5	0.69	HPLC
Rebaudioside F %	0.5-1.5	0.62	HPLC
Stevioside %	20.0-30.0	23.58	HPLC
DulcosideA %	0.3-1.0	0.44	HPLC
Rubusodide %	2.0-4.0	3.20	HPLC
Steviobiosjde %	0-1.0	0.89	HPLC
Sweetness times	280-320	300	
Loss on Drying (%)	≤4.00	3.41	CP/USP
Ash (%)	≤0.1	0.09	GB(1g/580C/2hrs)
PH (1% solution)	4.5-6.0	5.20	
Particle Size		100% pass 80	
Specific Optical Rotation	-30°~-38°	-34.5°	GB
Specific Absorbance	≤0.05	0.029	GB
Lead (ppm)	≤0.1	0.05	CP
Arsenic(ppm)	≤0.1	Below detection limit	CP
Cadmium (ppm)	≤0.1	Below detection limit	CP
Mercury (ppm)	≤0.1	0.05	CP
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Yeast&Mold(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP

Solvents

Methanol (ppm)	≤200	97	CP/USP
Ethanol (ppm)	≤5000	501	CP/USP

Package:20kg drum or carton (two l food grade bags inside)
Country of Original: China
Note:NON-GMO NON-ALLERGEN

INSPECTION:Jin Meng Xu **RECHECK: Bao Juan Peng**

Appendix 3 Analytical Chromatograms for Multiple Production Batches of SXY Stevia® Total Steviol Glycosides 95%

(b) (6)

Appendix 3.1 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 3.2 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 3.3 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 3.4 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 3.5 SXY Stevia® Total Steviol Glycosides 95% Batch

Appendix 3.1 SXY Stevia® Total Steviol Glycosides 95% Batch (b) (6)

HPLC Inspection Record

高效液相色谱法检验记录

total steviol glycocides95% Batch code 共 页 第 页

Test product name 检品名称	甜菊糖苷	批 号	20160913
Specification 规 格	10mg×2瓶/盒	chromatographic column 色谱柱	C18反相色谱柱
Equipment model/number 设备型号/编号	1220LC/XY-01-005	mobile phase 流动相	乙腈:磷酸缓冲液=32:68
Flow rate 流动相流速	1.0ml/min	sample size 进样量	20ul
Detection wavelength 检测波长	210nm	column temperature 柱 温	40℃
Balance model/number 天平型号/编号	AUW120D/XY-01-001	Temperature 温 度	22℃
Relative humidity 相对湿度	48%	Inspector 检验人	徐金明
Inspection Date 检验日期	2016年9月10日	Reviewer 复核人	彭保辉

Standard sample name 标准品名称:		含量 content:			批号 Batch number:					
Amount of test sample 供试品取样量 m_1 =	15.8 mg									
Amount of test sample 供试品取样量 m_2 =	22.06 mg									
Amount of standard sample 标准品取样量 m_s =	STV	9.69 mg			RA	7.93 mg				
Peak area of standard sample 标准品峰面积 S_s =	STV	1848.4			RA	1243.2				
	RD	RA	STV	RF	RC	DA	RUB	RB	STB	
Peak area of test sample 供试品峰面积 $S1_s$ =	1070	15054	769.4	17.0	83.9	11.9	93.0	54.8	28.0	
	RD	RA	STV	RF	RC	DA	RUB	RB	STB	
Peak area of test sample 供试品峰面积 $S2_s$ =	1412	21504	1095	25.70	90.43	15.50	130.4	73.3	39.0	

Calculation formula 计算公式:

$$\text{含量}(\%) = \frac{\text{样品峰面积 } S_s \cdot \text{标准品样量 } W_s}{\text{标准品峰面积 } S_s \cdot \text{样品样量 } W_s} \times 100\%$$

莱苞迪苷A含量（以干基计），按下式计算：

$$\text{R-A 含量} = \frac{m_R \cdot A_1}{m \cdot A_2} \times 1.00 \times 100\%$$

RA content (calculated as dry basis), calculate as below

RA content

$$\text{Content}(\%) = \frac{\text{Peak area of sample} \cdot \text{Amount of standard sample}}{\text{Peak area of standard sample} \cdot \text{Amount of test sample}} \times 100\%$$

Batch No: (b) (6)

The other kinds of 8 glycosides is as stevioside content (cacalate as a dry basis.
其他8种糖苷以甜菊苷含量（以干基计），分别按下式计算：

RD 含量= $\frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\%$ cacalate as to the following formula

STV 含量= $\frac{m_s \cdot A_S}{m \cdot A_s} \times 1.00 \times 100\%$

RF 含量= $\frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\%$

RC 含量= $\frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\%$

DA 含量= $\frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.96 \times 100\%$

RUB 含量= $\frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\%$

RB 含量= $\frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\%$

STB 含量= $\frac{m_s \cdot A_{sb}}{m \cdot A_s} \times 0.60 \times 100\%$

式中： (respectively correspond with)

A_a、A_b、A_c、A_d、A_f、A_{da}、A_{ru}、A_{sb}：分别对应 RA、STV、RB、RC、RD、RF、DA、RUB、STB 的峰面积；

(standard sample) A_R、A_S：为标准品 RA 和 STV 的峰面积；(standard solution)

m_R、m_S：为RA 和 STV 标准溶液中 RA 和 STV 的样量(以干基计算)，单位为毫克（mg）；

m：试样溶液中试样的样量(以干基计算)，单位为毫克（mg）； (sample amount)

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

(The amount of sample in the sample solution) (The unit is mongram)

(calculate testing sample) 计算供试品（1）

R-A 含量= $\frac{m_R \cdot A_a}{m \cdot A_R} \times 1.00 \times 100\% =$ $\frac{7.93 \times 15844}{12432 \times 1580} \times 1.00 \times 100\% = 64.28\%$

RD 含量= $\frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\% =$ $\frac{9.69 \times 1070}{18484 \times 158} \times 1.40 \times 100\% = 0.48\%$

STV 含量= $\frac{m_s \cdot A_S}{m \cdot A_s} \times 1.00 \times 100\% =$ $\frac{9.69 \times 7684}{18484 \times 158} \times 1.00 \times 100\% = 25.04\%$

RF 含量= $\frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\% =$ $\frac{9.69 \times 170}{18484 \times 158} \times 1.16 \times 100\% = 0.63\%$

RC 含量= $\frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\% =$ $\frac{9.69 \times 639}{18484 \times 158} \times 1.18 \times 100\% = 2.43\%$

DA 含量= $\frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\% =$ $\frac{9.69 \times 119}{18484 \times 158} \times 0.98 \times 100\% = 0.38\%$

RUB 含量= $\frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\% =$ $\frac{9.69 \times 935}{18484 \times 158} \times 0.8 \times 100\% = 2.40\%$

Batch No: (b) (6)

(content)

RB 含量 $= \frac{m_s \cdot A_o}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 548}{1848.4 \times 158} \times 1.0 \times 100\% = 2.16\%$

STB 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 286}{1848.4 \times 158} \times 0.8 \times 100\% = 1.77\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

(total content) $= 10.48 + 6.28 + 2.50 + 0.63 + 2.43 + 0.38 + 2.40 + 2.16 + 0.77\% = 95.56\%$

calculate testing sample

计算供试品（2）

R-A 含量 $= \frac{m_R \cdot A_o}{m \cdot A_R} \times 1.00 \times 100\% = \frac{7.93 \times 21436.30}{124320 \times 22.06} \times 1.0 \times 100\% = 61.07\%$

RD 含量 $= \frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\% = \frac{9.69 \times 1430}{1848.4 \times 22.06} \times 1.4 \times 100\% = 0.66\%$

STV 含量 $= \frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 5861.1}{1848.4 \times 22.06} \times 1.00 \times 100\% = 25.61\%$

RF 含量 $= \frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\% = \frac{9.69 \times 25.7}{1848.4 \times 22.06} \times 1.16 \times 100\% = 0.69\%$

RC 含量 $= \frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\% = \frac{9.69 \times 9048}{1848.4 \times 22.06} \times 1.18 \times 100\% = 2.46\%$

DA 含量 $= \frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\% = \frac{9.69 \times 153}{1848.4 \times 22.06} \times 0.98 \times 100\% = 0.35\%$

RUB 含量 $= \frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{4.69 \times 1324}{1848.4 \times 22.06} \times 0.8 \times 100\% = 2.44\%$

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 733}{1848.4 \times 22.06} \times 1.0 \times 100\% = 1.69\%$

STB 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 39.0}{1848.4 \times 22.06} \times 0.8 \times 100\% = 0.72\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= (0.46 + 61.07 + 25.61 + 0.69 + 2.46 + 0.35 + 2.44 + 1.69 + 0.72)\% = 95.49\%$

平均总含量(%)= 95.52%

Average Total Content

平均 RA 含量（%）= 61.18%

Average RA content

相对偏差（%）= 0.13%

Relative deviation

限度（%）≤1.5%

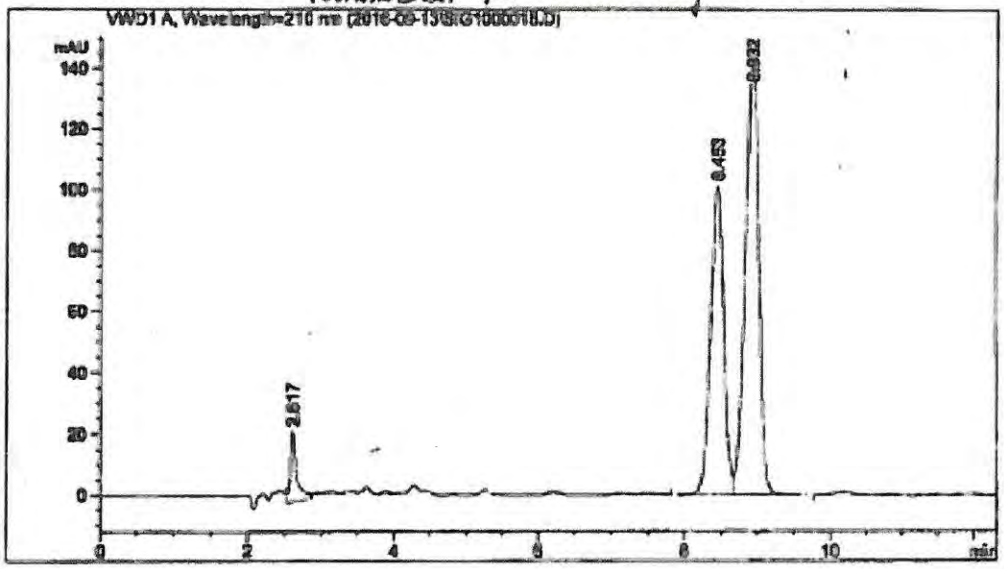
limit

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-13\SIG1000018.D
样品名称:
Sample name

操作者 operator :
仪器 instrument : 仪器 1
进样日期 Sample date : 2016-9-13 22:43:01
Position Sample bottle
位置 : 样品瓶 18
Sample Size
进样量 : 手动 manual

Collecting methods
采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
Last modified 最后修改 : 2016-9-13 22:25:37
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
Last modified 最后修改 : 2017-2-3 9:44:03
(调用后修改) invoked modification



面积百分比报告

排序 Sort
乘积因子: Product factor 信号 Signal : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
Internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm

peak #	峰	保留时间 [min]	类型 peak type	峰宽 peak width [min]	峰面积 peak area [mAU*s]	峰高 peak height [mAU]	峰面积 peak area %
1		2.617	VV	0.0825	135.16518	23.03171	4.1889
2		8.453	BV	0.1912	1243.21521	101.30580	38.5282
3		8.932	VB	0.2011	1848.38635	142.72313	57.2829

instrument
仪器 1 2017-2-3 9:45:31 页 1/2

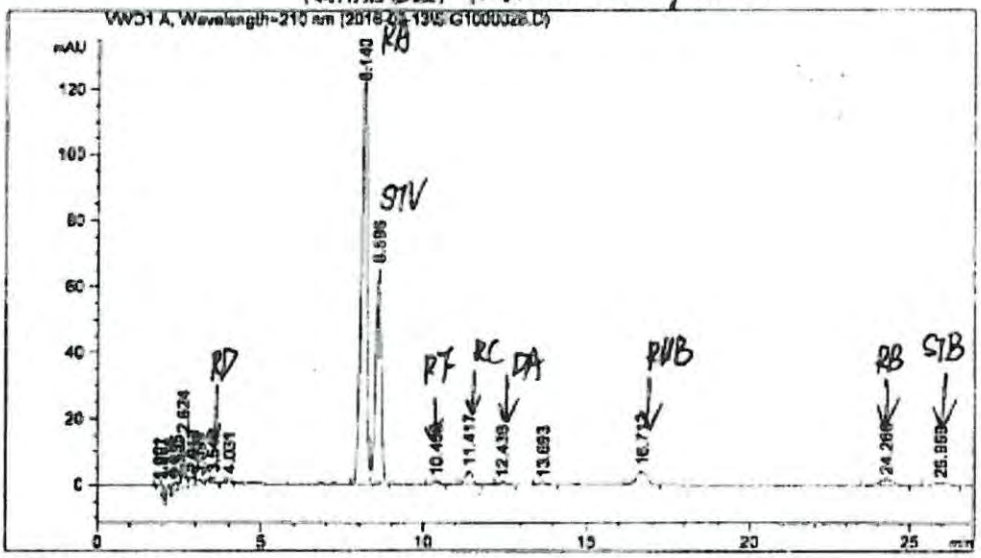
Batch No: (b) (6)

date file
数据文件: D:\CHEM32\1\DATA\2016-09-13\SIG1000028.D
样品名称:
Sample name

操作者 Operator :
仪器 Instrument 仪器 1
进样日期 Sample date : 2016-9-14 5:05:13

position Sample bottle
位置 : 样品瓶 28
Sample size
进样量 : 手动 manual

collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-14 5:03:21
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 17:21:57
(调用后修改) invoked modification



面积百分比报告

排序 Sort
乘积因子: product factor : 1.0000
稀释因子: diluting factor : 1.0000
信号 signal
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm
Peak retention time type peak width peak area peak height peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	1.901	VV	0.1035	19.03208	2.45425	0.6723
2	1.967	VB	0.0922	23.14308	3.28425	0.8176
3	2.216	BB	0.1486	27.96271	3.14755	0.9878
4	2.435	BV	0.1368	28.36320	2.67373	1.0020

仪器 1 2017-2-3 17:22:38 页 1/2

Instrument

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-13\SIG1000028.D
Sample name 样品名称:

peak 峰 #	保留时间 [min] (retention time)	类型 type	峰宽 [min] (peak width)	峰面积 [mAU*s] (peak area)	峰高 [mAU] (peak height)	峰面积 % (peak area)
5	2.624	VV	0.0662	73.33127	15.48972	2.5905
6	2.913	VV	0.1547	30.32296	2.66680	1.0712
7	3.099	VB	0.1531	11.02755	1.06205	0.3896
8	3.540	VB	0.1037	10.71674	1.54924	0.3786
9	4.031	VV	0.1340	11.35344	1.29808	0.4011
10	8.140	BV	0.1872	1545.38110	128.62016	54.5929
11	8.596	VB	0.1818	769.39471	65.15427	27.1800
12	10.400	BB	0.2210	16.99324	1.20142	0.6003
13	11.417	VB	0.2407	63.85032	4.07766	2.2556
14	12.436	VB	0.2420	11.86395	7.64293e-1	0.4191
15	13.693	VB	0.2655	11.45520	6.56658e-1	0.4047
16	16.712	VB	0.3231	93.11731	4.34338	3.2895
17	24.260	BB	0.4520	54.81236	1.79629	1.9363
18	25.969	BV	0.4072	28.61450	9.11737e-1	1.0109
总量 : total amount				2830.73562	241.13155	

••• 报告结束 •••
report end

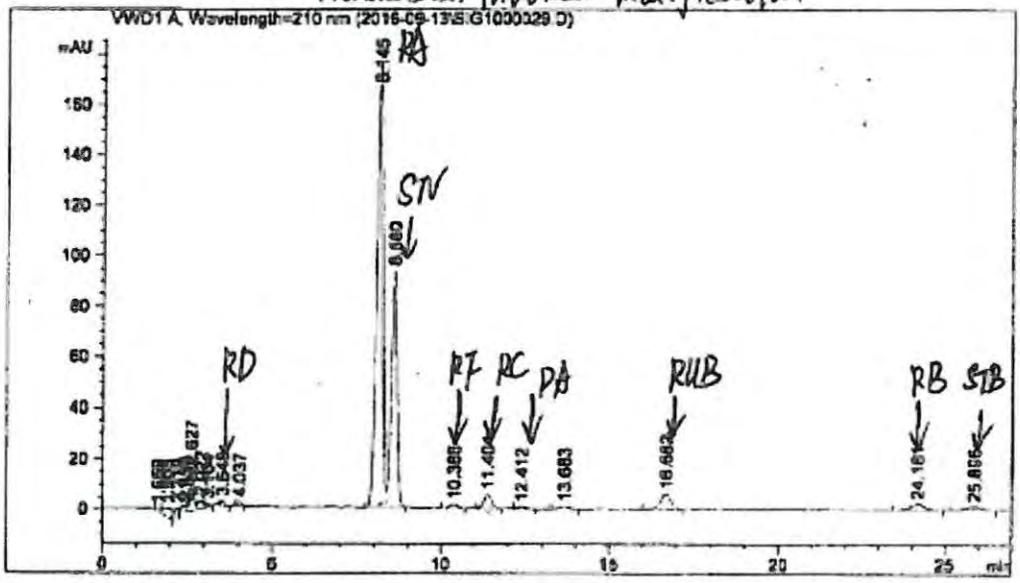
RA = 61.28%
RD = 0.48%
STV = 25.04%
RF = 0.63%
RC = 2.43%
DA = 0.38%
RNB = 2.40%
RB = 2.16%
STB = 0.77%

仪器 1 2017-2-3 17:22:38 页 2/2

Batch No: (b) (6)

date file
数据文件: D:\CHEM32\1\DATA\2016-09-13\SIG1000029.D
样品名称:
Sample name

operator
操作者 :
仪器 Instrument : 仪器 1
Position Sample bottle
位置 : 样品瓶 29
Sample date
进样日期 : 2016-9-14 5:36:54
Sample Spe
进样量 : 手动 manual
Collecting method
采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified
最后修改 : 2016-9-14 5:32:04
(调用后修改) invoked modification
analyce method
分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified
最后修改 : 2017-2-3 17:21:57
(调用后修改) invoked modification



面积百分比报告

排序 sort
信号 signal
乘积因子: product factor : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm

Peak 峰 #	Retention Time 保留时间 [min]	type 类型	Peak Width 峰宽 [min]	Peak Area 峰面积 [mAU*s]	peak height 峰高 [mAU]	Peak area 峰面积 %
1	1.659	VV	0.1477	16.19044	1.38171	0.4109
2	1.908	VB	0.2169	57.07411	3.33124	1.4486
3	2.219	BB	0.1503	28.10259	3.14454	0.7133
4	2.439	BV	0.1286	27.28297	2.77632	0.6925

instrument
仪器 1 2017-2-3 17:23:02
页 1/2

Batch No: (b) (6)

data file
Sample name 数据文件: D:\CHEM32\1\DATA\2016-09-13\SIG1000029.D
样品名称:
peak retention time type peak width peak area peak height peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
5	2.539	VV	0.0627	11.52531	2.54932	0.2925
6	2.627	VV	0.0690	79.76276	16.61504	2.0245
7	2.922	VV	0.1588	37.49392	3.19789	0.9517
8	3.104	VB	0.1522	13.68176	1.35000	0.3473
9	3.548	VB	0.1013	14.17116	2.11152	0.3597
10	4.037	VV	0.1341	15.15368	1.73160	0.3846
11	8.145	BV	0.1089	2150.35498	176.80806	54.5797
12	8.580	VB	0.1826	1095.06335	93.49480	27.7946
13	10.386	BB	0.2145	24.32161	1.75670	0.6173
14	11.404	VB	0.2437	89.75881	5.73202	2.2782
15	12.412	VB	0.2533	15.27640	8.94107e-1	0.3877
16	13.683	BV	0.2873	18.89295	1.02362	0.4795
17	16.682	BB	0.3324	132.41600	6.16866	3.3609
18	24.181	VV	0.4501	73.58027	2.40364	1.8676
19	25.896	VB	0.4012	39.74146	1.26959	1.0087
总量 :				3939.80454	327.74037	

total amount

*** 报告结束 ***
report end

RA = 61.07%
RD = 0.46%
STV = 25.61%
RT = 0.89%
RC = 2.46%
DA = 0.35%
RUB = 2.44%
RB = 1.69%
STB = 0.72%

Appendix 3.2 SXY Stevia® Total Steviol Glycosides 95% Batch (b) (6)

HPLC Inspection Record

高效液相色谱法检验记录

Test Product Name: total steviol glycosides95%　　共　页　第　页

检品名称	甜菊糖苷	批　号	20160914
规　格	10mg×2瓶/箱	色谱柱	C18反相色谱柱
设备型号/编号	D20LC/XY-01-005	流动相	乙腈:磷酸缓冲液=32:68
流动相流速	1ml/min	进样量	20μl
检测波长	210nm	柱　温	40℃
天平型号/编号	AUW120D/XY-01-001	温　度	21.2
相对湿度	48%	检验人	徐金明
检验日期	2016年9月14日	复核人	高多保

标准品名称：STV, RA.　含量：Content　批号：Batch Number

供试品取样量 m_{1a}	22.29mg								
供试品取样量 m_{1b}	24.4mg								
标准品取样量 m_{2}	STV	9.69mg			RA	7.93mg			
标准品峰面积 S_{2}	STV	1849			RA	123240			
供试品峰面积 $S1_a$	RD	RA	STV	RF	RC	DA	RUB	RB	STB
	24.00	240230	98370	25.50	81.20	15.20	118.70	24.40	31.10
供试品峰面积 $S2_a$	RD	RA	STV	RF	RC	DA	RUB	RB	STB
	23.0	24174	10151.50	26.20	87.0	20.10	127.60	83.2	30.2

Calculation formula 计算公式:

content 含量（%）$= \dfrac{\text{样品峰面积 } S_a \cdot \text{标准品样量 } W_S}{\text{标准品峰面积 } S_a \cdot \text{样品样量 } W_a} \times 100\%$

莱苞迪苷A含量（以干基计），按下式计算：

R-A 含量 $= \dfrac{m_R \cdot A_a}{m \cdot A_R} \times 1.00 \times 100\%$

RA content (calculated as dry basis), calculate as below

RA content

$$\text{Content (\%)} = \frac{\text{Peak area of sample} \cdot \text{Amount of standard sample}}{\text{Peak area of standard sample} \cdot \text{Amount of test sample}} \times 100\%$$

Batch No: (b) (6)

The other kind of 8 glycosides is as stevioside content (caculated as a dry basis)

其他8种糖苷以甜菊苷含量（以干基计），分别按下式计算：

Caculate as to the following formula

RD 含量=$\frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\%$

STV 含量=$\frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\%$

RF 含量=$\frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\%$

RC 含量=$\frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\%$

DA 含量=$\frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\%$

RUB 含量=$\frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\%$

RB 含量=$\frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\%$

STB 含量=$\frac{m_s \cdot A_{sb}}{m \cdot A_s} \times 0.80 \times 100\%$

式中：（respectively correspond with）（peak area）

A_{RA}、A_{STV}、A_{RB}、A_{RC}、A_{RD}、A_{RF}、A_{DA}、A_{RUB}、A_{STB}：分别对应RA、STV、RB、RC、RD、RF、DA、RUB、STB的峰面积；

（Standard sample）A_{RA}、A_{ST}：为标准品RA和STV的峰面积；→（Standard solution）

m_R、m_{ST}：为RA和STV标准溶液中RA和STV的样量(以干基计算)，单位为毫克（mg）；

m：试样溶液中试样的样量(以干基计算)，单位为毫克（mg）；（Sample amount）

总含量(%)=RD+RA+STV+RF+RC+DA+RUB+RB+STB

（The amount of sample in the sample solution）（The unit is milligram）

（Calculate testing sample）计算供试品（1）

RA 含量=$\frac{m_R \cdot A_s}{m \cdot A_R} \times 1.00 \times 100\%$= $\frac{7.93 \times 2202.30}{1232.4 \times 22.29} \times 1.0 \times 100\% = 63.58\%$

RD 含量=$\frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\%$= $\frac{9.69 \times 21.30}{1849.0 \times 22.29} \times 1.40 \times 100\% = 0.71\%$

STV 含量=$\frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\%$= $\frac{9.69 \times 9832.70}{1849.0 \times 22.29} \times 1.0 \times 100\% = 23.13\%$

RF 含量=$\frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\%$= $\frac{9.69 \times 25.30}{1849.0 \times 22.29} \times 1.16 \times 100\% = 0.59\%$

RC 含量=$\frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\%$= $\frac{9.69 \times 81.30}{1849.0 \times 22.29} \times 1.18 \times 100\% = 1.91\%$

DA 含量=$\frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\%$= $\frac{9.69 \times 15.30}{1849.0 \times 22.29} \times 0.98 \times 100\% = 0.36\%$

RUB 含量=$\frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\%$= $\frac{9.69 \times 1118.70}{1849.0 \times 22.29} \times 0.80 \times 100\% = 2.79\%$

Batch No: (b) (6)

(content)

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 74.40}{1849.0 \times 32.29} \times 1.0 \times 100\% = 1.75\%$

STB 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 31.10}{1849.0 \times 22.39} \times 0.80 \times 100\% = 0.73\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

(total content) $= 0.71\% + 63.58\% + 23.13\% + 0.59\% + 1.91\% + 0.36\% + 2.79\% + 1.75\% + 0.73\% = 95.54\%$

(Calculate testing sample) 计算供试品 (2)

R-A 含量 $= \frac{m_R \cdot A_s}{m \cdot A_R} \times 1.00 \times 100\% = \frac{9.69 \times 22.0}{1849.0 \times 24.45} \times \frac{7.93 \times 2487.4}{1224 \times 24.45} \times 1.0 \times 100\% = 68$

RD 含量 $= \frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\% = \frac{9.69 \times 22.0}{1849.0 \times 24.45} \times 1.40 \times 100\% = 0.69\%$

STV 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 1081.5}{1849.0 \times 24.45} \times 1.0 \times 100\% = 23.18\%$

RF 含量 $= \frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\% = \frac{9.69 \times 26.20}{1849.0 \times 24.45} \times 1.16 \times 100\% = 0.56\%$

RC 含量 $= \frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\% = \frac{9.69 \times 870}{1849.0 \times 24.45} \times 1.18 \times 100\% = 1.86\%$

DA 含量 $= \frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\% = \frac{9.69 \times 20.81}{1849.0 \times 24.45} \times 0.98 \times 100\% = 0.43\%$

RUB 含量 $= \frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 127.6}{1849.0 \times 24.45} \times 0.80 \times 100\% = 2.74\%$

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 83.20}{1849.0 \times 24.45} \times 1.0 \times 100\% = 1.78\%$

STB 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 34.20}{1849.0 \times 24.45} \times 0.80 \times 100\% = 0.78\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= 0.69\% + 63.62\% + 23.18\% + 0.56\% + 1.86\% + 0.43\% + 2.74\% + 1.78\% + 0.73\% = 95.60\%$

平均总含量(%)= 95.57%
(Average Total Content)

平均RA含量 (%) = 63.60%
(Average RA Content)

相对偏差 (%) = 0.03%
(Relative deviation)

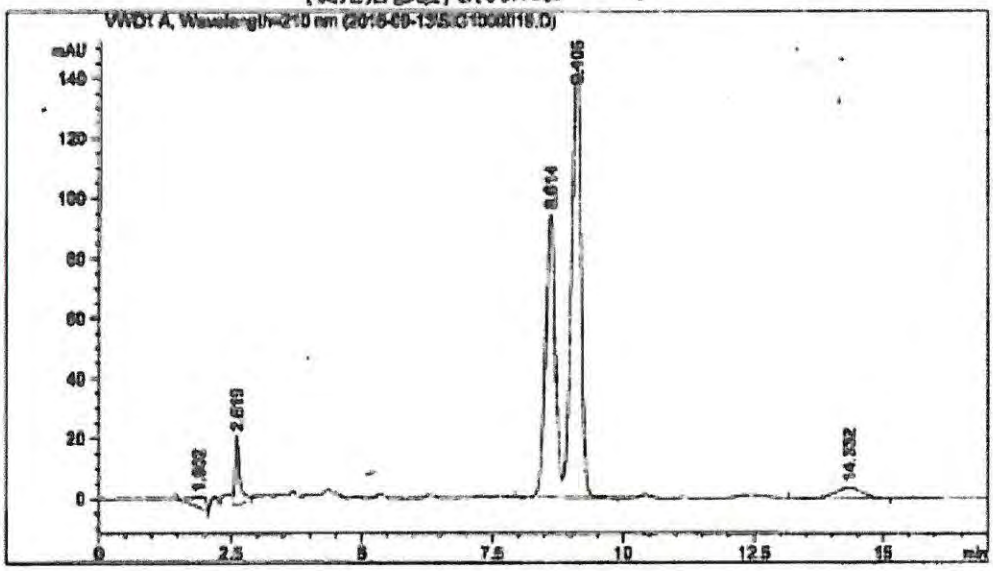
限度 (%) ≤1.5%
(Limit)

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-13\SIG1000019.D
样品名称:
Sample name

操作者 operator :
仪器 instrument: 仪器 1
进样日期 Sample date: 2016-9-13 22:56:36
采集方法 collecting methods
最后修改 last modified: D:\CHEM32\1\METHODS\TIANJUTANG.M
分析方法 analyse methods
最后修改 last modified: 2016-9-13 22:55:26
(调用后修改) invoked modification
D:\CHEM32\1\METHODS\TIANJUTANG.M
2017-2-3 9:58:03
(调用后修改) invoked modification

position sample bottle
位置 : 样品瓶 19
进样量 : 手动 manual
Sample size



面积百分比报告 area percentage report

排序 sort
乘积因子: product factor : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor
信号 Signal

信号 1: VWD1 A, Wavelength=210 nm
peak remain time type peak width peak area peak height peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	1.902	BB	0.2773	79.42109	3.51882	2.3219
2	2.619	VV	0.0839	135.91682	22.69116	3.9735
3	8.614	BV	0.2045	1232.44092	94.31576	36.0300
4	9.106	VB	0.1991	1848.95642	144.64856	54.0537

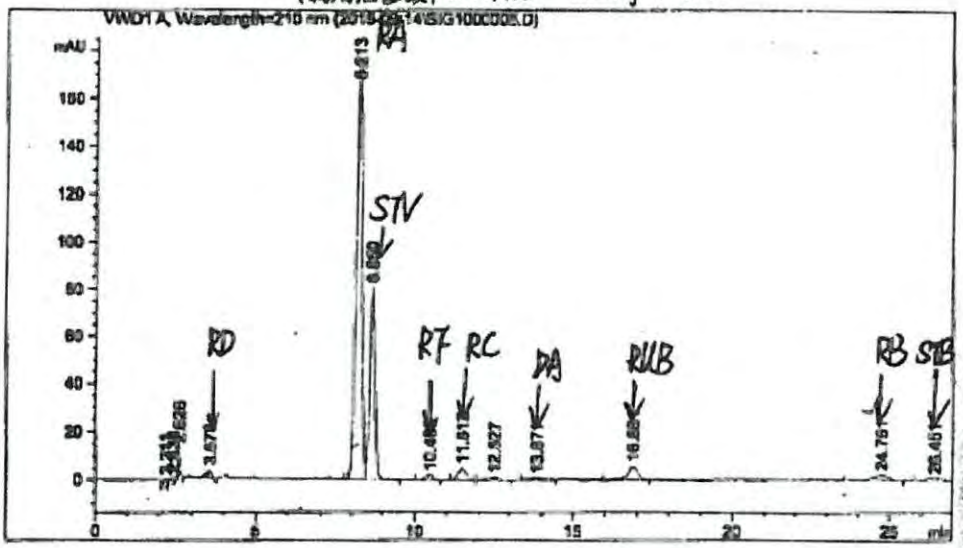
仪器 1 2017-2-3 10:01:58
instrument

页 1/2
page

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-14\SIG1000008.D
样品名称:
sample name

operator 操作者 :
instrument 仪器 : 仪器 1 instument
sample date 进样日期 : 2016-9-14 23:57:02
position sample bottle 位置 : 样品瓶 8
sample size 进样量 : 手动 manual
collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-14 23:55:46
(调用后修改) invoked modification
analysis methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 17:05:46
(调用后修改) invoked modification



面积百分比报告 area percentage report

排序 sort 信号 signal
乘积因子: product factor : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

signal 信号 1: VWD1 A, Wavelength=210 nm
peak remain time type peak width peak area peak height peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	2.211	BB	0.1468	28.23843	3.20455	0.7675
2	2.438	BV	0.1486	25.76010	2.23284	0.7001
3	2.626	VB	0.0601	53.50341	13.53582	1.4541
4	3.578	VB	0.0977	21.56220	3.41111	0.5860

仪器 1 2017-2-3 17:05:51 页 1/2
instrument page

Batch No: (b) (6)

data file

数据文件: D:\CHEM32\1\DATA\2016-09-14\SIG1000008.D
样品名称:

Sample name — Peak retention time — type — peak width — peak area — peak height — peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
5	8.213	BV	0.1937	2202.37354	176.33093	59.8571
6	8.650	VB	0.1884	983.77460	80.59255	26.7375
7	10.486	BV	0.2327	25.31071	1.69051	0.6879
8	11.512	VB	0.2670	81.28195	4.76537	2.2091
9	12.527	BB	0.3038	17.97243	8.60075e-1	0.4885
10	13.871	BB	0.2962	15.29352	7.12990e-1	0.4157
11	16.881	BB	0.3221	118.73099	5.53760	3.2269
12	24.761	BB	0.5033	74.40431	2.15236	2.0222
13	26.451	BBA	0.4504	31.17725	9.61157e-1	0.8473
总量：				3679.38343	295.98787	

total amount

*** 报告结束 ***
report end

RA = 63.58%
RD = 0.71%
STV = 23.8%
RF = 0.59%
RC = 1.91%
DA = 0.36%
RUB = 2.79%
RB = 1.75%
STB = 0.73%

仪器 1 2017-2-3 17:05:51
Instrument

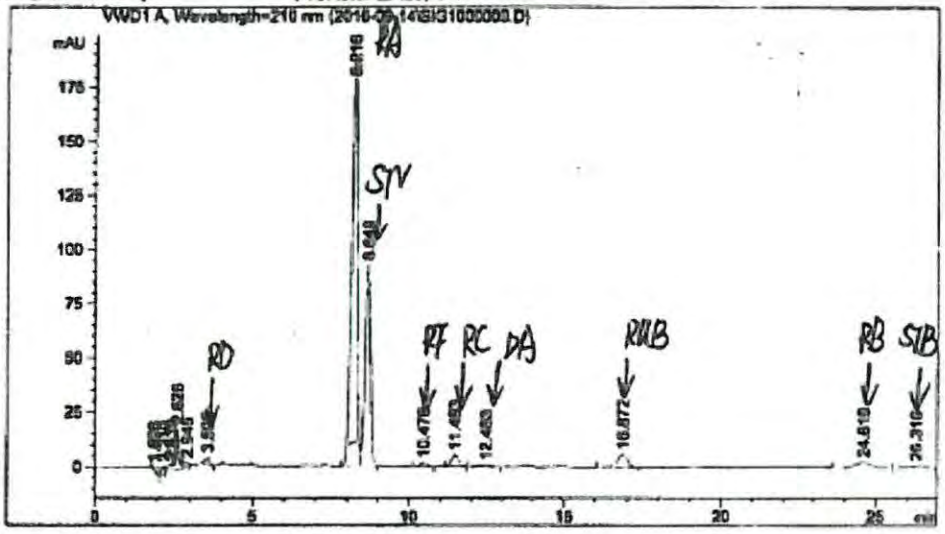
页 2/2

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-14\SIG1000009.D
样品名称:
sample name

操作者 operator :
仪器 instrument : 仪器 1 instrument
进样日期 sample date : 2016-9-15 0:24:43
采集方法 collecting methods : D:\CHEM32\1\METHODS\TIANJUTANG.M
最后修改 last modified : 2016-9-15 0:23:53
(调用后修改) invoked modification
分析方法 analyse methods : D:\CHEM32\1\METHODS\TIANJUTANG.M
最后修改 last modified : 2017-2-3 17:00:27
(调用后修改) invoked modification

position sample bottle
位置 : 样品瓶 9
进样量 : 手动 manual
sample size



面积百分比报告 area percentage report

排序 sort
乘积因子: product factor 信号 signal : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子 interdnal standard use product factor and diluting factor

信号 1: VWD1 A, Wavelength=210 nm
signal
peak remain time type peak width peak area peak height peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	1.896	BB	0.2321	29.62646	1.59927	0.7205
2	2.210	BB	0.1463	28.26587	3.22319	0.6874
3	2.439	BV	0.1400	30.76406	2.84891	0.7482
4	2.626	VV	0.0726	93.92701	18.69099	2.2844

仪器 1 2017-2-3 17:00:29
instrument

页 1/2
page

Batch No: (b) (6)

datafile

Sample name

数据文件: D:\CHEM32\1\DATA\2016-09-14\SIG1000009.D
样品名称:

peak / retention time / type / peak width / peak area / peak height / peak area

峰	保留时间	类型	峰宽	峰面积	峰高	峰面积
#	[min]		[min]	[mAU*s]	[mAU]	%
5	2.945	VV	0.1712	28.96812	2.17803	0.7045
6	3.595	VB	0.0950	23.03133	3.68206	0.5601
7	8.216	BV	0.1990	2417.41992	189.33557	58.7930
8	8.649	VV	0.1822	1081.56775	92.65084	26.3043
9	10.476	BB	0.2190	26.16330	1.87290	0.6363
10	11.483	VB	0.2511	87.00121	5.36855	2.1159
11	12.483	BB	0.3181	20.10167	9.08586e-1	0.4889
12	16.872	BB	0.3322	127.56060	5.90131	3.1023
13	24.619	BB	0.4995	83.17353	2.55692	2.0228
14	26.316	BBA	0.4414	34.17686	1.04539	0.8312
总量:				4111.74769	331.86252	

total amount

*** 报告结束 ***
report and

RA = 63.63%
RD = 0.69%
STV = 23.18%
RF = 0.56%
RC = 1.86%
DA = 0.43%
RVB = 2.74%
RB = 1.78%
STB = 0.78%

Appendix 3.3 SXY Stevia® Total Steviol Glycosides 95% Batch (b) (6)

HPLC Inspection Record

高效液相色谱法检验记录

total steviol glycocides95% batch code 共 页 第 页

Test product Name 检品名称	甜菊糖苷	批 号	20160916
Specification 规 格	10g×2袋/盒	Chromatographic column 色谱柱	C18反相色谱柱
Equipment model /number 设备型号/编号	1220HC/XY-01-005	moving phase 流动相	乙腈:还原型的缓冲液=23:77
Flow rate 流动相流速	1ml/min	sample size 进样量	20ul
Detection wavelength 检测波长	21nm	column temperature 柱 温	40℃
Balance model /number 天平型号/编号	AUW120D/XY-01-007	Temperature 温 度	20℃
Relative humidity 相对湿度	48%	Inspector 检验人	徐金明
Inspection Date 检验日期	2016年09月15日	Reviewer 复核人	赵保力

Standard sample name 标准品名称: STV, RA. Content 含量: Batch Number 批号:

Amount of test sample 供试品取样量 m_1	17.56mg								
Amount of test sample 供试品取样量 m_2	17.02mg								
Amount of standard sample 标准品取样量 m_R	STV	9.69mg			RA	7.93mg			
Peak area of standard sample 标准品峰面积 S_R	STV	18103			RA	1208			
	RD	RA	STV	RF	RC	DA	RUB	RB	STB
Peak area of test sample 供试品峰面积 $S1_n$	8.10	163.2	8248	19.70	60.88	11.30	10.10	53.9	29.0
	RD	RA	STV	RF	RC	DA	RUB	RB	STB
Peak area of test sample 供试品峰面积 $S2_n$	7.5	158.9	807.1	18.2	59.4	10.33	9.17	53.3	29.5

Calculation formula 计算公式:

$$含量(\%)=\frac{样品峰面积\ S_n \cdot 标准品样量\ W_R}{标准品峰面积\ S_R \cdot 样品样量\ W_n}\times 100\%$$

莱苞迪苷A含量（以干基计），按下式计算：

$$R\text{-}A\ 含量=\frac{m_R \cdot A_n}{m \cdot A_R}\times 1.00\times 100\%$$

RA content

RA content (calculated as dry basis), calculate as below

$$Content(\%)=\frac{Peak\ area\ of\ sample \cdot Amount\ of\ standard\ sample}{Peak\ area\ of\ standard\ sample \cdot Amount\ of\ test\ sample}\times 100\%$$

Batch No: (b) (6)

The other kind of 8 glycosides is as steviboside content (caculated as a dry basis)

其他8种糖苷以甜菊苷含量（以干基计），分别按下式计算：

Caculate as to the following formula

$$\text{RD 含量}=\frac{m_s\cdot A_R}{m\cdot A_s}\times 1.40\times 100\%$$

$$\text{STV 含量}=\frac{m_s\cdot A_S}{m\cdot A_s}\times 1.00\times 100\%$$

$$\text{RF 含量}=\frac{m_s\cdot A_F}{m\cdot A_s}\times 1.16\times 100\%$$

$$\text{RC 含量}=\frac{m_s\cdot A_C}{m\cdot A_s}\times 1.18\times 100\%$$

$$\text{DA 含量}=\frac{m_s\cdot A_D}{m\cdot A_s}\times 0.98\times 100\%$$

$$\text{RUB 含量}=\frac{m_s\cdot A_U}{m\cdot A_s}\times 0.80\times 100\%$$

$$\text{RB 含量}=\frac{m_s\cdot A_B}{m\cdot A_s}\times 1.00\times 100\%$$

$$\text{STB 含量}=\frac{m_s\cdot A_D}{m\cdot A_s}\times 0.80\times 100\%$$

式中：

A_R、A_S、A_B、A_C、A_F、A_D、A_U、A_D：分别对应RA、STV、RB、RC、RF、DA、RUB、STB的峰面积；

（respectively correspond with）（peak area）

（Starbard sample）A_R、A_S：为标准品RA和STV的峰面积；→（Standard solution）

m_R、m_S：为RA和STV标准溶液中RA和STV的样量(以干基计算)，单位为毫克（mg）；

m：试样溶液中试样的样量(以干基计算)，单位为毫克（mg）；→（sample amount）

总含量(%)=RD+RA+STV+RF+RC+DA+RUB+RB+STB

（The amount of sample is the sample solution（The unit is milligram）

计算供试品（1）

（Calculate tating sample）

$$\text{R-A 含量}=\frac{m_R\cdot A_s}{m\cdot A_R}\times 1.00\times 100\%=\frac{7.93\times 1132.60}{1208.00\times 1756}\times 100\times 100\%=61.11\%$$

$$\text{RD 含量}=\frac{m_s\cdot A_R}{m\cdot A_s}\times 1.40\times 100\%=\frac{7.69\times 8.10}{1810.30\times 1756}\times 1.40\times 100\%=0.85\%$$

$$\text{STV 含量}=\frac{m_s\cdot A_S}{m\cdot A_s}\times 1.00\times 100\%=\frac{7.69\times 5282.93}{1810.30\times 1756}\times 1.00\times 100\%=12.42\%$$

$$\text{RF 含量}=\frac{m_s\cdot A_F}{m\cdot A_s}\times 1.16\times 100\%=\frac{9.69\times 13.20}{1810.30\times 1756}\times 1.16\times 100\%=0.60\%$$

$$\text{RC 含量}=\frac{m_s\cdot A_C}{m\cdot A_s}\times 1.18\times 100\%=\frac{9.69\times 2088}{1810.30\times 1756}\times 1.18\times 100\%=1.86\%$$

$$\text{DA 含量}=\frac{m_s\cdot A_D}{m\cdot A_s}\times 0.98\times 100\%=\frac{9.69\times 11.30}{1810.30\times 1756}\times 0.98\times 100\%=0.34\%$$

$$\text{RUB 含量}=\frac{m_s\cdot A_U}{m\cdot A_s}\times 0.80\times 100\%=\frac{9.69\times 101.00}{1810.30\times 1756}\times 0.80\times 100\%=3.08\%$$

Batch No: (b) (6)

Content

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 5386}{1870.30 \times 17.56} \times 1.00 \times 100\% = 1.44\%$

STB 含量 $= \frac{m_s \cdot A_{sb}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 28.00}{1870.30 \times 17.56} \times 0.80 \times 100\% = 0.88\%$

Total content 总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= (0.33 + 61.24 + 25.42 + 0.60 + 1.86 + 0.54 + 3.08 + 1.44 + 0.88)\% = 95.28\%$

(Caculate testing sample)

计算供试品 (Σ)

R-A 含量 $= \frac{m_R \cdot A_b}{m \cdot A_R} \times 1.00 \times 100\% = \frac{7.93 \times 1591.90}{1208.00 \times 17.02} \times 1.00 \times 100\% = 61.21\%$

RD 含量 $= \frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\% = \frac{9.69 \times 7.50}{1208.00 \times 17.02} \times 1.40 \times 100\% = 0.33\%$

STV 含量 $= \frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 807.10}{1208.00 \times 17.02} \times 1.00 \times 100\% = 25.38\%$

RF 含量 $= \frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\% = \frac{9.69 \times 18.20}{1208.00 \times 17.02} \times 1.16 \times 100\% = 0.57\%$

RC 含量 $= \frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\% = \frac{9.69 \times 59.46}{1208.00 \times 17.02} \times 1.18 \times 100\% = 1.87\%$

DA 含量 $= \frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\% = \frac{9.69 \times 10.33}{1208.00 \times 17.02} \times 0.98 \times 100\% = 0.32\%$

RUB 含量 $= \frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 97.17}{1208.00 \times 17.02} \times 0.80 \times 100\% = 3.06\%$

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 52.30}{1208.00 \times 17.02} \times 1.00 \times 100\% = 1.65\%$

STB 含量 $= \frac{m_s \cdot A_{sb}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 29.51}{1208.00 \times 17.02} \times 0.80 \times 100\% = 0.94\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= (0.33 + 61.24 + 25.38 + 0.57 + 1.87 + 0.32 + 3.06 + 1.65 + 0.94)\% = 95.36\%$

平均总含量(%)= 95.32%

Average Total Content

相对偏差 (%) = 0.09%

Relative deviation

平均 RA 含量 (%) = 61.16%

Average RA content

限度 (%) ≤1.5%

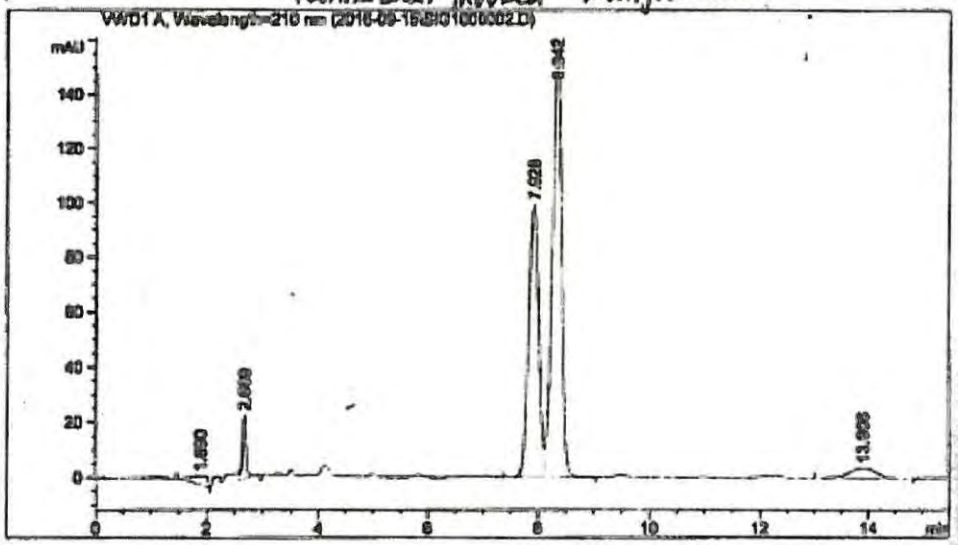
Limit

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000002.D
样品名称:
Sample name

操作者 operator:
仪器 Instrument: 仪器 1
进样日期 Sample date: 2016-9-15 8:26:39
位置 Sample bottle position: 样品瓶 2
进样量 Sample size: 手动 manual

采集方法 Collecting methods: D:\CHEM32\1\METHODS\TIANJUTANG.M
最后修改 last modified: 2016-9-15 8:25:38
(调用后修改) invoked modification
分析方法 analyse methods: D:\CHEM32\1\METHODS\TIANJUTANG.M
最后修改 last modified: 2017-2-3 9:53:09
(调用后修改) invoked modification



面积百分比报告

排序 Sort
信号 Signal
乘积因子 Product factor: 1.0000
稀释因子 diluting factor: 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm

Peak 峰 #	Retention time 保留时间 [min]	Type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area 峰面积 %
1	1.890	BB	0.2687	69.81305	3.24936	2.1025
2	2.669	VB	0.0666	103.95596	23.06825	3.1308
3	7.928	BV	0.1901	1208.03149	99.20332	36.3818
4	8.342	VB	0.1842	1810.39355	152.88324	54.5229

仪器 1 2017-2-3 9:53:18
Instrument

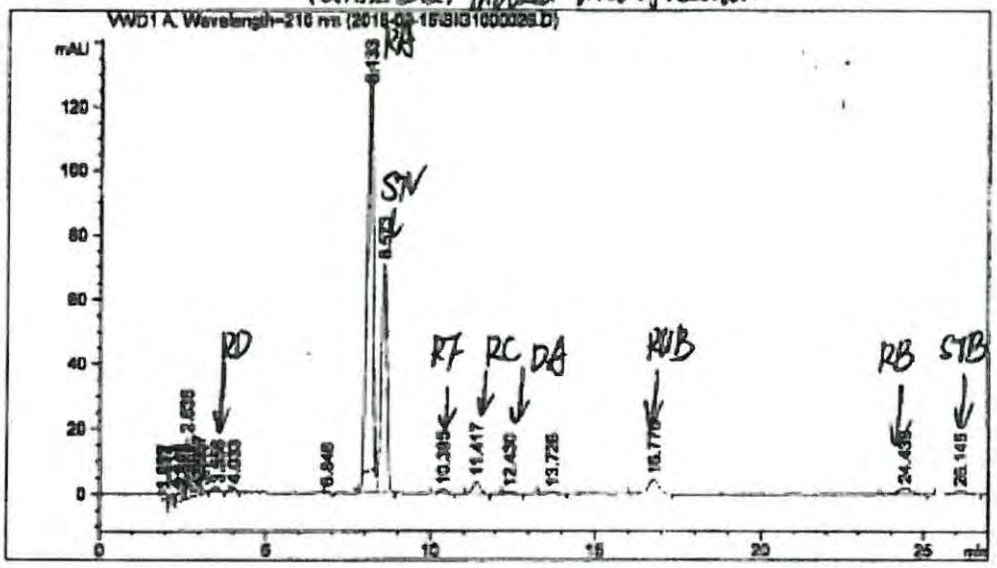
页 1/2

Batch No: (b) (6)

date file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000026.D
样品名称:
Sample name

操作者 operator :
仪器 Instrument : 仪器 1
进样日期 Sample date : 2016-9-15 21:03:08
position Sample bottle
位置 : 样品瓶 26
Sample size
进样量 : 手动 manual

Collecting method
采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
Last modified 最后修改 : 2016-9-15 21:01:56
(调用后修改) invoked modification
analyse methods
分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
Last modified 最后修改 : 2017-2-3 17:31:16
(调用后修改) invoked modification



面积百分比报告

排序 Sort
乘积因子: Product factor : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
Internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm

Peak 峰 #	retention time 保留时间 [min]	Type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	Peak area 峰面积 %
1	1.897	BV	0.0815	9.21108	1.54562	0.3082
2	1.972	VB	0.1062	18.93722	2.78856	0.6336
3	2.213	BB	0.1509	28.73175	3.22544	0.9613
4	2.442	BV	0.1390	28.35592	2.62494	0.9487

仪器 1 2017-2-3 17:31:31 页 1/2
instrument

Batch No: (b) (6)

date file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000026.D

Sample name 样品名称:

Peak 峰 #	retention time 保留时间 [min]	type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area 峰面积 %
5	2.528	VV	0.0680	10.98441	2.33170	0.3675
6	2.635	VV	0.0682	86.74949	18.66723	2.9024
7	2.861	VV	0.0907	8.62503	1.40260	0.2886
8	2.987	VV	0.0888	21.97006	3.42834	0.7350
9	3.117	VB	0.0876	6.16563	1.03363	0.2063
10	3.556	VB	0.1058	8.10952	1.15619	0.2713
11	4.033	VV	0.1268	10.20545	1.24224	0.3414
12	6.868	VB	0.1309	5.01196	5.79339e-1	0.1677
13	8.133	BV	0.1894	1631.22803	133.68457	54.5757
14	8.573	VB	0.1025	824.83112	70.51795	27.5962
15	10.395	BV	0.2310	19.67913	1.28969	0.6584
16	11.417	VB	0.2517	61.85176	3.84476	2.0694
17	12.430	VB	0.2656	11.25627	6.23637e-1	0.3766
18	13.726	VB	0.3009	12.60643	6.05390e-1	0.4218
19	16.770	VB	0.3486	101.03731	4.45551	3.3804
20	24.439	VB	0.4747	54.17265	1.76203	1.8124
21	26.145	VV	0.4389	29.20932	9.31333e-1	0.9773
总量 :				2988.92950	257.74071	

total amount

*** 报告结束 ***
report end

RA = 61.11%
RD = 0.35%
GTV = 25.42%
RT = 0.60%
RE = 1.86%
PB = 0.34%
PUB = 3.08%
PB = 1.64%
STB = 0.88%

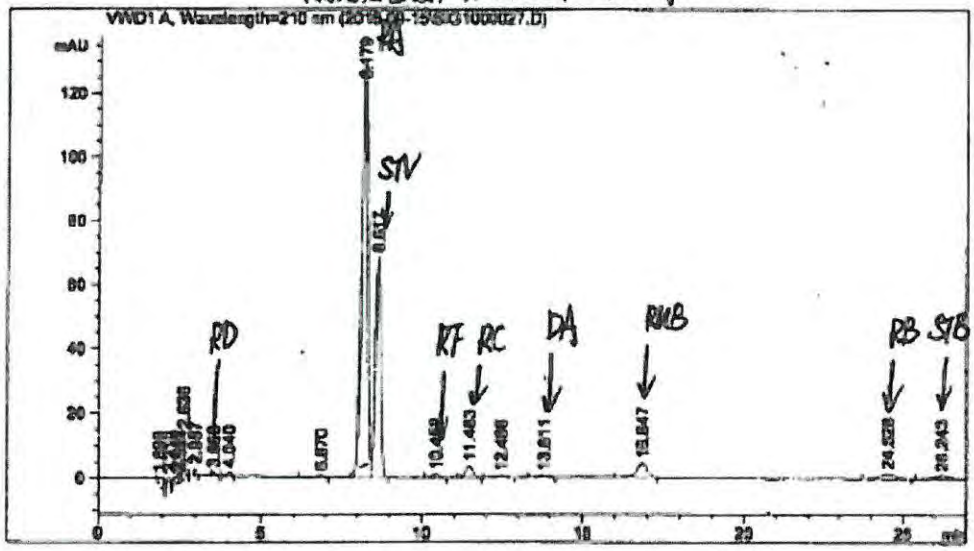
仪器 1 2017-2-3 17:31:31 页 2/2
Instrument

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000027.D
样品名称:
Sample name

操作者 operator :
仪器 instrument : 仪器 1
进样日期 Sample date : 2016-9-15 21:32:12
Position Sample bottle
位置 : 样品瓶 27
Sample size
进样量 : 手动 manual

Collecting method
采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modification 最后修改 : 2016-9-15 21:30:00
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 16:58:32
(调用后修改) invoked modification



面积百分比报告

排序 sort
信号 signal
乘积因子: Product factor : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm

Peak #	Retention time 保留时间 [min]	Type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area % 峰面积 %
1	1.899	BB	0.2167	31.53968	1.83317	1.1003
2	2.210	BB	0.1516	28.88103	3.25919	1.0079
3	2.442	BV	0.1469	24.34868	2.10590	0.8494
4	2.521	VV	0.0666	7.11254	1.57608	0.2481

仪器 1 2017-2-3 16:58:37 页 1/2
instrument

Batch No: (b) (6)

date file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000027.D

Sample name ← 样品名称:

Peak 峰 #	retention time 保留时间 [min]	type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area 峰面积 %
5	2.636	VB	0.0608	57.31411	14.29409	1.9994
6	2.987	BV	0.0702	10.94045	2.26931	0.3817
7	3.558	VB	0.1053	7.45843	1.07063	0.2602
8	4.040	BV	0.1529	11.95507	1.15381	0.4170
9	6.870	BV	0.2460	11.26185	6.08246e-1	0.3929
10	8.179	BV	0.1896	1586.91528	130.79402	55.3591
11	8.617	VV	0.1835	807.05322	68.49237	28.1539
12	10.452	BV	0.2403	18.24831	1.18110	0.6366
13	11.483	VB	0.2500	59.37105	3.68498	2.0711
14	12.486	BB	0.3159	13.40927	6.06638e-1	0.4678
15	13.811	BB	0.2876	10.33754	5.59613e-1	0.3606
16	16.847	BB	0.3372	97.17534	4.40732	3.3899
17	24.528	BB	0.4838	53.30056	1.69077	1.8594
18	26.243	BBA	0.4846	29.95939	9.37992e-1	1.0451
总量 :				2866.58178	240.52703	

total amount

••• 报告结束 •••
report end

RA = 61.21%
RD = 0.33%
STV = 25.38
RT = 0.57%
RC = 1.87%
DA = 0.32%
RUB = 3.06%
RB = 1.68%
STB = 0.94%.

instrument
仪器 1 2017-2-3 16:58:37

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Appendix 3.4 SXY Stevia® Total Steviol Glycosides 95% Batch (b) (6)

HPLC Inspection Record

高效液相色谱法检验记录

total steviol glycocides95% 共 页 第 页

Test product name 检品名称	甜菊糖苷	Batch Code 批号	20160917
Specification 规格	40吨X袋1箱	Chromatographic column 色谱柱	C18反相色谱柱
Equipment model /number 设备型号/编号	1220LCXY-01-005	Mobile phase 流动相	乙腈:磷酸缓冲液=32:68
Flow rate 流动相流速	1.0ml/min	Sample size 进样量	20ul
Detection wavelength 检测波长	210nm	Column temperature 柱温	40℃
Balance model /number 天平型号/编号	AUW120D XY-01-001	Temperature 温度	20℃
Relative humidity 相对湿度	47%	Inspector 检验人	徐金明
Inpection Date 检验日期	2016年09月16日	Reviewer 复核人	彭保锋

Standard sample name 标准品名称: STV, A3 含量: Content 批号: Batch number

Amount of test sample 供试品取样量 $m_{i样}$	23.2 mg								
Amount of test sample 供试品取样量 $m_{i标}$	18.83 mg								
Amount of standard sample 标准品取样量 m_{s}	STV	9.69			RA	7.93			
Peak area of standard sample 标准品峰面积 S_{s}	STV	20683			RA	13810			
	RD	RA	STV	RF	RC	DA	RUB	RB	STB
Peak area of test sample 供试品峰面积 S1 #	2414	25195	11778	28.6	90.1	19.9	107.3	83.3	48.5
	RD	RA	STV	RF	RC	DA	RUB	RB	STB
Peak area of test sample 供试品峰面积 S2 #	19.5	20077	9847	21.5	71.3	13.2	112.9	71.2	35.5

Calculation formula 计算公式:

$$含量(\%)=\frac{样品峰面积\ S_{样}\cdot 标准品样量\ W_{标}}{标准品峰面积\ S_{标}\cdot 样品样量\ W_{样}}\times 100\%$$

莱苞迪苷A含量（以干基计），按下式计算：

$$R\text{-}A\ 含量=\frac{m_{标}\cdot A_{样}}{m_{样}\cdot A_{标}}\times 1.00\times 100\%$$

RA content (calculated as dry basis), calculate as below

RA content

$$Content(\%)=\frac{Peak\ area\ of\ test\ sample\cdot Amount\ of\ standard\ sample}{Peak\ area\ of\ standard\ Sample\cdot Amount\ of\ test\ sample}\times 100\%$$

Batch No: (b) (6)

The other kinds of 8 glycosides is as steviocide content (calculated as a dry basis)
其他8种糖苷以甜菊苷含量（以干基计），分别按下式计算：

calculate as to the following formula

RD 含量=$\frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\%$

STV 含量=$\frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\%$

RF 含量=$\frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\%$

RC 含量=$\frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\%$

DA 含量=$\frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\%$

RUB 含量=$\frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\%$

RB 含量=$\frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\%$

STB 含量=$\frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\%$

式中：

A_{RA}、A_{RD}、A_{STV}、A_{RB}、A_{RC}、A_{RD}、A_{RF}、A_{DA}、A_{RUB}、A_{STB}：分别对应RA、STV、RB、RC、RD、RF、DA、RUB、STB的峰面积；

A_{RA}、A_{ST}：为标准品RA和STV的峰面积；

m_{RA}、m_{ST}：为RA和STV标准溶液中RA和STV的样量(以干基计算)，单位为毫克（mg）；

m：试样溶液中试样的样量(以干基计算)，单位为毫克（mg）；

总含量(%)=RD+RA+STV+RF+RC+DA+RUB+RB+STB

respectively correspond with peak area
standard sample
standard solution
sample component
The amount of sample in the sample solution
The unit is milligram

计算供试品（1）

calculate testing sample

RA 含量=$\frac{m_R \cdot A_R}{m \cdot A_R} \times 1.00 \times 100\% = \frac{7.93 \times 7519.5}{1380 \times 23.2} \times 1.0 \times 100\% = 62.55\%$

RD 含量=$\frac{m_s \cdot A_d}{m \cdot A_s} \times 1.40 \times 100\% = \frac{9.69 \times 24.6}{2068.3 \times 23.2} \times 1.4 \times 100\% = 0.69\%$

STV 含量=$\frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 1178}{2068.3 \times 23.2} \times 1.0 \times 100\% = 2.42\%$

RF 含量=$\frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\% = \frac{9.69 \times 28.4}{2068.3 \times 23.2} \times 1.16 \times 100\% = 0.58\%$

RC 含量=$\frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\% = \frac{9.69 \times 96.0}{2068.3 \times 23.2} \times 1.18 \times 100\% = 1.82\%$

DA 含量=$\frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\% = \frac{9.69 \times 19.9}{2068.3 \times 23.2} \times 0.98 \times 100\% = 0.40\%$

RUB 含量=$\frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 147.6}{2068.3 \times 23.2} \times 0.8 \times 100\% = 2.91\%$

Batch No: (b) (6)

content

RB 含量 $= \frac{m_s \cdot A_i}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 83.3}{2068.3 \times 23.2} \times 1.00 \times 100\% = 1.68\%$

STB 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 48.5}{2068.3 \times 23.2} \times 0.8 \times 100\% = 0.98\%$

total content 总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= 0.69\% + 62.55\% + 24.20\% + 0.58\% + 1.82\% + 0.40\% + 2.91\% + 1.68\% + 0.98\% = 95.81\%$

caculate testing sample) 计算供试品 (2)

R-A 含量 $= \frac{m_R \cdot A_s}{m \cdot A_R} \times 1.00 \times 100\% = \frac{7.93 \times 2007}{1980 \times 18.43} \times 1.0 \times 100\% = 62.60\%$

RD 含量 $= \frac{m_s \cdot A_i}{m \cdot A_s} \times 1.40 \times 100\% = \frac{9.69 \times 19.5}{2068.3 \times 18.43} \times 1.4 \times 100\% = 0.69\%$

STV 含量 $= \frac{m_s \cdot A_s}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 9446.7}{2068.3 \times 18.43} \times 1.0 \times 100\% = 24.0\%$

RF 含量 $= \frac{m_s \cdot A_f}{m \cdot A_s} \times 1.16 \times 100\% = \frac{9.69 \times 21.5}{2068.3 \times 18.43} \times 1.16 \times 100\% = 0.55\%$

RC 含量 $= \frac{m_s \cdot A_c}{m \cdot A_s} \times 1.18 \times 100\% = \frac{9.69 \times 71.3}{2068.3 \times 18.43} \times 1.18 \times 100\% = 1.81\%$

DA 含量 $= \frac{m_s \cdot A_{da}}{m \cdot A_s} \times 0.98 \times 100\% = \frac{9.69 \times 15.2}{2068.3 \times 18.43} \times 0.98 \times 100\% = 0.34\%$

RUB 含量 $= \frac{m_s \cdot A_{ru}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 112.9}{2068.3 \times 18.43} \times 0.8 \times 100\% = 2.87\%$

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 7.2}{2068.3 \times 18.43} \times 1.00 \times 100\% = 1.91\%$

STB 含量 $= \frac{m_s \cdot A_{st}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 35.5}{2068.3 \times 18.43} \times 0.8 \times 100\% = 0.9\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= 0.69\% + 62.6\% + 24.0\% + 0.55\% + 1.81\% + 0.34\% + 2.87\% + 0.9\%$

$= 95.58\%$

平均总含量(%)= 95.70%
(Average Total content)

平均 RA 含量 (%) = 62.58%
(Average RA content)

相对偏差 (%) = 0.17%
(Relative deviation)

限度 (%) ≤1.5%
(limit)

Batch No: (b) (6)

Data file
数据文件: D:\CHEM32\1\DATA\2016-09-16\SIG1000001.D
Sample name 样品名称:

operator
instrument 仪器 : 仪器 1
sample data 进样日期 : 2016-9-16 9:03:43
collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-16 9:00:02 (调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 10:02:16 (调用后修改) invoked modification

position sample bottle
位置 : 样品瓶 1
sample size
进样量 : 手动 manual

面积百分比报告

排序 Sort
乘积因子: product factor : 1.0000
稀释因子: diluting factor : 1.0000
信号 Signal
内标使用乘积因子和稀释因子

internal standard use product factor and diluting factor

信号 1: VWD1 A, Wavelength=210 nm

Peak 峰 #	Retention time 保留时间 [min]	Type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area 峰面积 %
1	2.621	VV	0.0792	133.43713	23.87642	3.7255
2	7.919	BV	0.1809	1380.04651	119.35974	38.5298
3	8.380	VV	0.1779	2068.28125	180.26134	57.7448

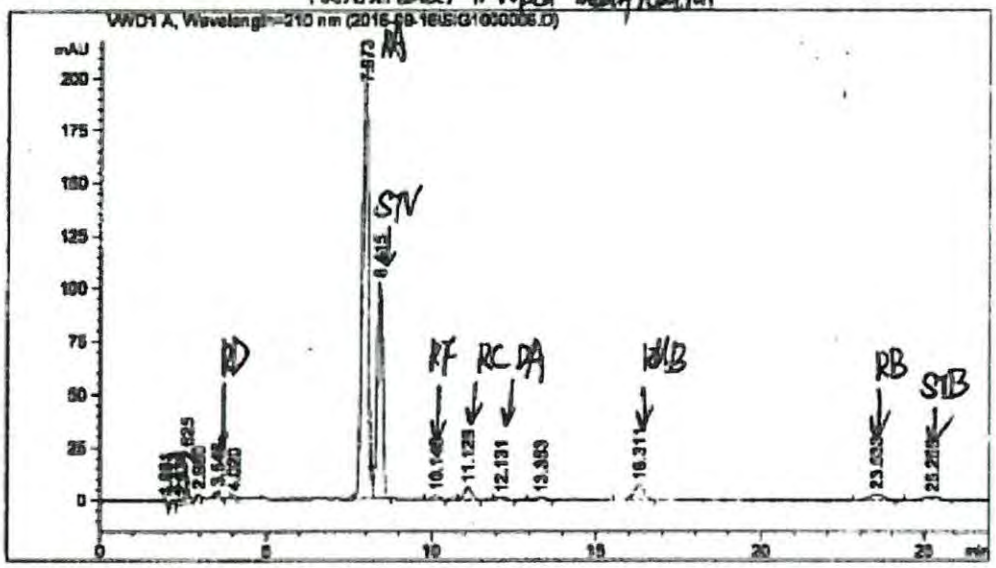
Instrument
仪器 1 2017-2-3 10:02:33

Batch No: (b) (6)

Data file 数据文件: D:\CHEM32\1\DATA\2016-09-16\SIG1000006.D
sample name 样品名称:

operator 操作者 :
instrument 仪器 : 仪器 1
sample data 进样日期 : 2016-9-16 11:29:08
collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-16 11:24:35
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 17:23:44
(调用后修改) invoked modification

position 位置 : 样品瓶 6 sample bottle
sample size 进样量 : 手动 manual



面积百分比报告

排序 sort
乘积因子: product factor 信号 signal : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

信号 1: VWD1 A, Wavelength=210 nm

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	1.981	VB	0.1017	20.21572	3.11404	0.4631
2	2.213	BB	0.1494	30.79010	3.50960	0.7054
3	2.434	BV	0.1340	31.80152	3.06609	0.7285
4	2.625	VV	0.0707	90.06750	18.54283	2.0634

仪器 1 2017-2-3 17:23:48 页 1/2
instrument.

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-16\SIG1000006.D
Sample name 样品名称: retention time type peak width peak area peak height peak area

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
5	2.980	VV	0.0825	20.33351	3.46103	0.4658
6	3.546	VB	0.0960	24.36497	3.89629	0.5582
7	4.020	VV	0.1384	15.40338	1.70424	0.3529
8	7.973	BV	0.1894	2519.48267	207.85840	57.7188
9	8.415	VB	0.1796	1177.77734	102.08598	26.9817
10	10.146	BV	0.2174	28.62420	2.04405	0.6558
11	11.126	VB	0.2418	90.07287	5.78059	2.0635
12	12.131	VB	0.2517	19.90892	1.19284	0.4561
13	13.353	VB	0.2855	16.94677	8.92559e-1	0.3882
14	16.311	VB	0.3252	147.61945	7.08034	3.3818
15	23.533	VV	0.4514	83.23116	2.83594	1.9067
16	25.255	BB	0.4732	48.45619	1.52708	1.1101
总量 :				4365.09627	369.59188	

total amount

*** 报告结束 ***
report end.

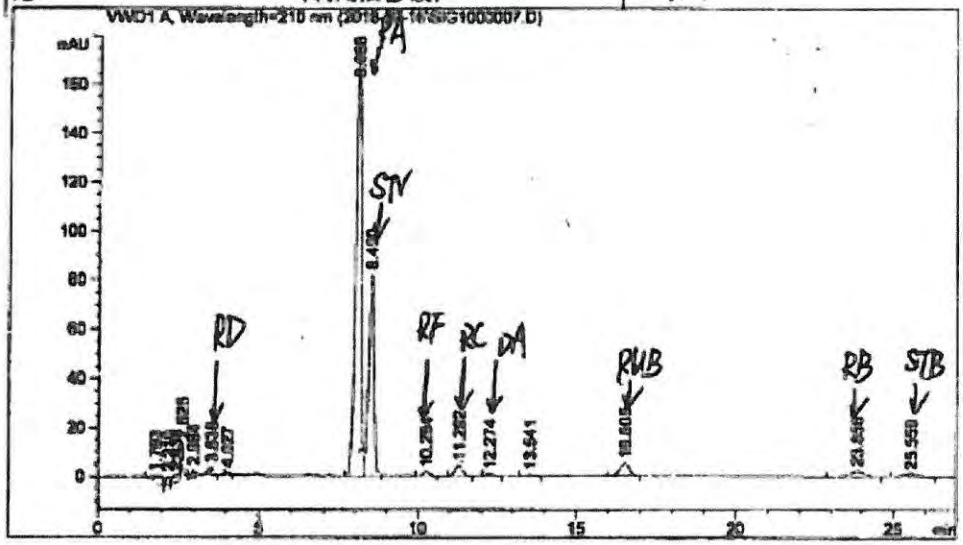
RA = 62.55%
RD = 0.69%
STV = 24.20%
RF = 0.58%
RE = 1.92%
DA = 0.40%
RUB = 2.91%
RB = 1.68%
STB = 0.98%

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-16\SIG1000007.D
Sample name 样品名称:

operator 操作者 :
Instrument 仪器 : 仪器 1
Sample data 进样日期 : 2016-9-16 12:29:37
Collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-16 11:55:59
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 17:08:26
(调用后修改) invoked modification

position 位置 : 样品瓶 7 sample bottle
sample one 进样盘 : 手动 manual



面积百分比报告

排序 sort
乘积因子: product factor : 1.0000
稀释因子: diluting factor : 1.0000
信号 signal
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

信号 1: VWD1 A, Wavelength=210 nm
signal

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	1.793	BB	0.3636	71.17318	2.37500	1.9942
2	2.210	BB	0.1496	30.73086	3.52887	0.8611
3	2.436	BV	0.1020	43.69794	3.03445	1.2244
4	2.625	VV	0.0732	77.85779	15.34483	2.1815

peak 峰 retention Time 保留时间 type 类型 peak width 峰宽 peak area 峰面积 peak height 峰高 peak area 峰面积

仪器 1 2017-2-3 17:08:39 页 1/2

instrument

Batch No: (b) (6)

(1)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-16\SIG1000007.D

Sample name 样品名称:

peak 峰 #	retention time 保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
5	2.984	VV	0.1105	24.94129	3.01383	0.6988
6	3.538	VB	0.0965	19.53126	3.14125	0.5473
7	4.027	VV	0.1411	11.07626	1.22956	0.3104
8	8.066	BV	0.1031	2007.78894	170.87325	56.2574
9	8.490	VV	0.1787	944.71021	81.81865	26.4704
10	10.264	BB	0.2173	21.56663	1.55997	0.6043
11	11.292	BV	0.2538	71.32577	4.38327	1.9985
12	12.274	VB	0.2570	13.25960	7.93479e-1	0.3715
13	13.541	BB	0.2706	11.63195	6.69904e-1	0.3259
14	16.505	BB	0.3216	112.89748	5.45214	3.1633
15	23.856	BB	0.4872	71.24409	2.28878	1.9962
16	25.559	BB	0.4684	35.50055	1.13655	0.9947
总量 :				3568.93381	300.64377	

total amount

*** 报告结束 ***
report end

RA = 62.60%

RD = 0.69%

STV = 24.20%

RF = 0.58%

RC = 1.82%

PA = 0.40%

RUB = 2.91%

RB = 1.68%

STB = 0.98%

仪器 1 2017-2-3 17:08:39 頁 2/2

Appendix 3.5 SXY Stevia® Total Steviol Glycosides 95% Batch (b) (6)

HPLC Inspection Record

高效液相色谱法检验记录

total steviol glycocides95% Batch code 共 页 第 页

Test product name	检品名称	甜菊提取物	批 号	20160918
specification	规 格	10kg×2/桶	色谱柱	C18 反相色谱柱
Equipment model/number	设备型号/编号	1220LC/XY-01-005	流动相	乙腈:磷酸盐缓冲液=32:68
Flow rate	流动相流速	1.0ml/min	进样量	20μL
Detection wavelength	检测波长	210nm	柱温	40℃
Balance model/number	天平型号/编号	BSW120B/XY-01-001	温 度	21℃
Relative humidity	相对湿度	49%	检验人	徐金盟
Inspection Date	检验日期	2016年9月16日	复核人	彭保锦

Standard sample name: 标准品名称: 甜菊提取物 含量: content 95% 批号: Batch code 20160918

Amount of test sample	供试品取样量 m_1	18.11mg							
Amount of test sample	供试品取样量 m_2	19.80mg							
Amount of standard sample	标准品取样量 m_x	STV 959mg				RA 793mg			
Peak area of standard sample	标准品峰面积 S_x	STV 18104				RA 12014			
Peak area of test sample	供试品峰面积 $S1_n$	RD	RA	STV	RF	RC	DA	RUB	RB	STB
		16.6	17779	7951	21.0	678	168	1089	595	30.7
Peak area of test sample	供试品峰面积 $S2_n$	RD	RA	STV	RF	RC	DA	RUB	RB	STB
		182	18729	8749	228	748	1444	1176	69.8	327

Calculation formula 计算公式:

$$\text{含量（\%）} = \frac{\text{样品峰面积 } S_n \cdot \text{标准品样量 } W_s}{\text{标准品峰面积 } S_x \cdot \text{样品样量 } W_n} \times 100\%$$

莱苞迪苷 A 含量（以干基计），按下式计算：

$$\text{R-A 含量} = \frac{m_s \cdot A_n}{m \cdot A_s} \times 1.00 \times 100\%$$

RA content (calculated as dry basis), calculate as below

RA content

$$\text{Content (\%)} = \frac{\text{Peak area of sample} \cdot \text{Amount of standard sample}}{\text{Peak area of standard sample} \cdot \text{Amount of test sample}} \times 100\%$$

Batch No: (b) (6)

The other kind of 9 glycosides is as stevioside content (caculated as a dry basis)
其他8种甜苷以甜茶苷含量（以干基计），分别按下式计算：

Caculate as to the following formula)

$$\text{RD 含量}=\frac{m_s\cdot A_d}{m\cdot A_s}\times 1.40\times 100\%$$

$$\text{STV 含量}=\frac{m_s\cdot A_s}{m\cdot A_s}\times 1.00\times 100\%$$

$$\text{RF 含量}=\frac{m_s\cdot A_f}{m\cdot A_s}\times 1.16\times 100\%$$

$$\text{RC 含量}=\frac{m_s\cdot A_c}{m\cdot A_s}\times 1.18\times 100\%$$

$$\text{DA 含量}=\frac{m_s\cdot A_{da}}{m\cdot A_s}\times 0.98\times 100\%$$

$$\text{RUB 含量}=\frac{m_s\cdot A_{ru}}{m\cdot A_s}\times 0.80\times 100\%$$

$$\text{RB 含量}=\frac{m_s\cdot A_b}{m\cdot A_s}\times 1.00\times 100\%$$

$$\text{STB 含量}=\frac{m_s\cdot A_{sb}}{m\cdot A_s}\times 0.80\times 100\%$$

式中：（respectively correspond with）（peak area）

A_d、A_s、A_f、A_c、A_{da}、A_{ru}、A_b、A_{sb}：分别对应RA、STV、RB、RC、RD、RF、DA、RUB、STB的峰面积；

A_R、A_S：为标准品RA和STV的峰面积；（Standard solution）

（Standard sample）m_R、m_S：为RA和STV标准溶液中RA和STV的样量(以干基计算)，单位为毫克（mg）；

m：试样溶液中试样的样量(以干基计算)，单位为毫克（mg）；（sample amount）

总含量(%)=RD+RA+STV+RF+RC+DA+RUB+RB+STB

（The amount of sample in the sample solution）（The unit is milligram）

计算供试品（1）

（Caculate testing sample）

$$\text{R-A 含量}=\frac{m_{sa}\cdot A_a}{m\cdot A_{sa}}\times 1.00\times 100\%=\frac{7.93\times 171290}{1210.4\times 18.11}\times 1.00\times 100\%=62.61\%$$

$$\text{RD 含量}=\frac{m_s\cdot A_d}{m\cdot A_s}\times 1.40\times 100\%=\frac{9.69\times 16.60}{1810.4\times 18.11}\times 1.40\times 100\%=0.69\%$$

$$\text{STV 含量}=\frac{m_s\cdot A_s}{m\cdot A_s}\times 1.00\times 100\%=\frac{9.69\times 79.80}{1810.4\times 18.11}\times 1.00\times 100\%=2.34\%$$

$$\text{RF 含量}=\frac{m_s\cdot A_f}{m\cdot A_s}\times 1.16\times 100\%=\frac{9.69\times 21.0}{1810.4\times 18.11}\times 1.16\times 100\%=0.62\%$$

$$\text{RC 含量}=\frac{m_s\cdot A_c}{m\cdot A_s}\times 1.18\times 100\%=\frac{9.69\times 67.80}{1810.4\times 18.11}\times 1.18\times 100\%=2.00\%$$

$$\text{DA 含量}=\frac{m_s\cdot A_{da}}{m\cdot A_s}\times 0.98\times 100\%=\frac{9.69\times 16.80}{1810.4\times 18.11}\times 0.98\times 100\%=0.50\%$$

$$\text{RUB 含量}=\frac{m_s\cdot A_{ru}}{m\cdot A_s}\times 0.80\times 100\%=\frac{9.69\times 106.90}{1810.4\times 18.11}\times 0.80\times 100\%=3.32\%$$

Batch No (b) (6)

(content)

RB 含量 $= \frac{m_s \cdot A_b}{m \cdot A_s} \times 1.00 \times 100\% = \frac{9.69 \times 59.50}{1810.4 \times 19.11} \times 1.00 \times 100\% = 1.76\%$

STB 含量 $= \frac{m_s \cdot A_{ab}}{m \cdot A_s} \times 0.80 \times 100\% = \frac{9.69 \times 32.70}{1810.4 \times 19.11} \times 0.80 \times 100\% = 0.91\%$

(total content) 总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= 0.69\% + 62.64\% + 23.65\% + 0.62\% + 2.02\% + 0.39\% + 3.18\% + 1.76\% + 0.91\% = 95.80\%$

(testing sample) 计算供试品 (2)

R-A 含量 $= \frac{m_R \cdot A_b}{m \cdot A_R} \times 1.00 \times 100\% = \frac{7.93 \times 1878.9}{1201.4 \times 19.80} \times 1.00 \times 100\% = 62.64\%$

RD 含量 $= \frac{m_R \cdot A_b}{m \cdot A_R} \times 1.40 \times 100\% = \frac{9.69 \times 18.20}{1810.4 \times 19.80} \times 1.4 \times 100\% = 0.69\%$

STV 含量 $= \frac{m_R \cdot A_S}{m \cdot A_R} \times 1.00 \times 100\% = \frac{9.69 \times 874.90}{1810.4 \times 19.80} \times 1.00 \times 100\% = 23.65\%$

RF 含量 $= \frac{m_R \cdot A_F}{m \cdot A_R} \times 1.16 \times 100\% = \frac{9.69 \times 22.80}{1810.4 \times 19.80} \times 1.16 \times 100\% = 0.62\%$

RC 含量 $= \frac{m_R \cdot A_C}{m \cdot A_R} \times 1.18 \times 100\% = \frac{9.69 \times 74.80}{1810.4 \times 19.80} \times 1.18 \times 100\% = 2.02\%$

DA 含量 $= \frac{m_R \cdot A_{da}}{m \cdot A_R} \times 0.98 \times 100\% = \frac{9.69 \times 14.40}{1810.4 \times 19.80} \times 0.98 \times 100\% = 0.39\%$

RUB 含量 $= \frac{m_R \cdot A_{ru}}{m \cdot A_R} \times 0.80 \times 100\% = \frac{9.69 \times 117.60}{1810.4 \times 19.80} \times 0.80 \times 100\% = 3.18\%$

RB 含量 $= \frac{m_R \cdot A_b}{m \cdot A_R} \times 1.00 \times 100\% = \frac{9.69 \times 69.75}{1810.4 \times 19.80} \times 1.00 \times 100\% = 1.89\%$

STB 含量 $= \frac{m_R \cdot A_{ab}}{m \cdot A_R} \times 0.80 \times 100\% = \frac{9.69 \times 32.70}{1810.4 \times 19.80} \times 0.80 \times 100\% = 0.88\%$

总含量(%)=RD+ RA+ STV+ RF+ RC+ DA+ RUB+ RB+ STB

$= 0.69\% + 62.64\% + 23.65\% + 0.62\% + 2.02\% + 0.39\% + 3.18\% + 1.89\% + 0.88\% = 95.95\%$

平均总含量(%)= 95.88%

(Average Total Content)

相对偏差 (%) =0.10%

(Relative deviation)

平均 RA 含量 (%) = 62.63%

(Average RA content)

限度 (%) ≤1.5%

limit

Batch No (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000002.D
样品名称:
Sample name

操作者 operator :
仪器 instrument : 仪器 1
进样日期 Sample date 2016-9-15 8:26:39
position Sample bottle
位置 : 样品瓶 2
Sample Size
进样量 : 手动 manual

Collecting methods
采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-15 8:25:38
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 9:37:55
(调用后修改) invoked modification

面积百分比报告

排序 Sort
乘积因子: product factor 信号 Signal : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
Internal standard use product factor and diluting factor

Signal 信号 1: VWD1 A, Wavelength=210 nm

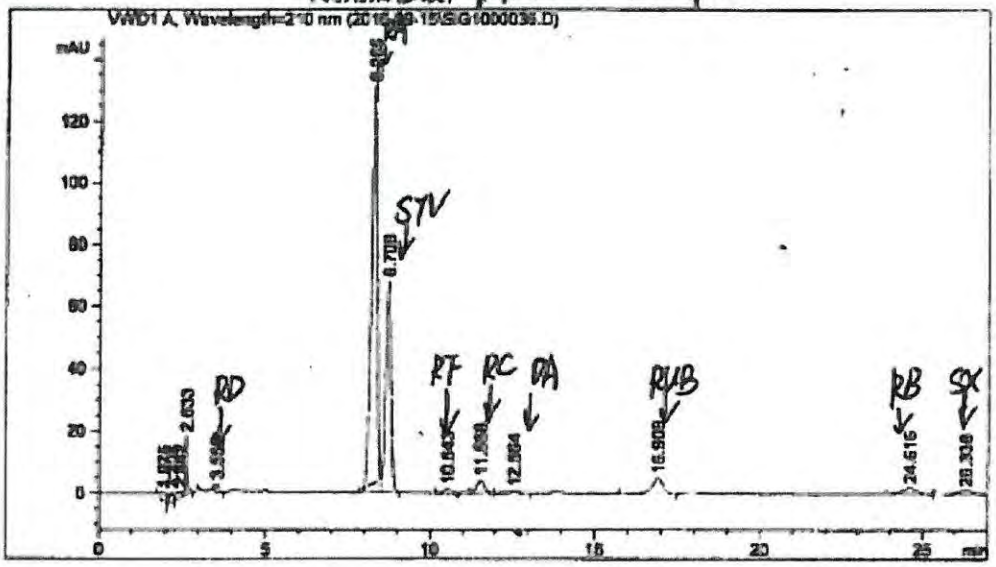
Peak 峰 #	retetim 保留时间 [min]	tmd type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	peak area 峰面积 %
1	1.890	BB	0.2687	69.81305	3.24936	2.1025
2	2.669	VB	0.0666	103.95596	23.06825	3.1308
3	7.928	BV	0.1901	1208.03149	99.20332	36.3818
4	8.342	VB	0.1842	1810.39355	152.88324	54.5229

仪器 1 2017-2-3 9:38:08 页 1/2
Instrument

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000036.D
样品名称:
Sample name

操作者 operator :
仪器 Instrument : 仪器 1
Sample date 进样日期 : 2016-9-16 2:59:30
Postion Sample bottle 位置 : 样品瓶 36
Sample size 进样量 : 手动 manual
collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-16 2:57:48 (调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 16:57:24 (调用后修改) invoked modification



面积百分比报告

排序 sort
乘积因子: Product factor 信号 signal : 1.0000
稀释因子: diluting factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor

信号 1: VWD1 A, Wavelength=210 nm

Peak 峰 #	retention time 保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	1.975	BB	0.1659	38.69061	3.22551	1.2892
2	2.208	BB	0.1485	28.60555	3.25596	0.9531
3	2.442	BV	0.1455	24.61035	2.15078	0.8200
4	2.633	VB	0.0605	74.92482	18.78139	2.4965

仪器 1 2017-2-3 16:57:25 页 1/2
instrument

Batch No: (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000036.D

Sample name
样品名称:

Peak # 峰	Retention time 保留时间 [min]	Type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area 峰面积 %
5	3.552	VB	0.1053	16.59717	2.44191	0.5530
6	8.265	BV	0.1897	1717.92590	139.51393	57.2414
7	8.708	VB	0.1835	795.06433	67.46497	26.4916
8	10.543	BV	0.2269	21.01895	1.41839	0.7004
9	11.958	VB	0.2475	67.80385	4.26528	2.2592
10	12.564	BB	0.3120	16.86294	7.86830e-1	0.5619
11	16.909	BV	0.3365	108.90145	4.95291	3.6286
12	24.616	BB	0.4809	59.47458	1.92258	1.9817
13	26.338	BBA	0.5222	30.71407	9.18412e-1	1.0234
总量 :				3001.19458	251.09885	

total amount

*** 报告结束 ***
report end

RA = 62.61%
RD = 0.69%
STV = 23.50%
RF = 0.62%
RC = 2.00%
DA = 0.50%
RUB = 3.22%
RB = 1.76%
STB = 0.91%

仪器 1 2017-2-3 16:57:25
instrument

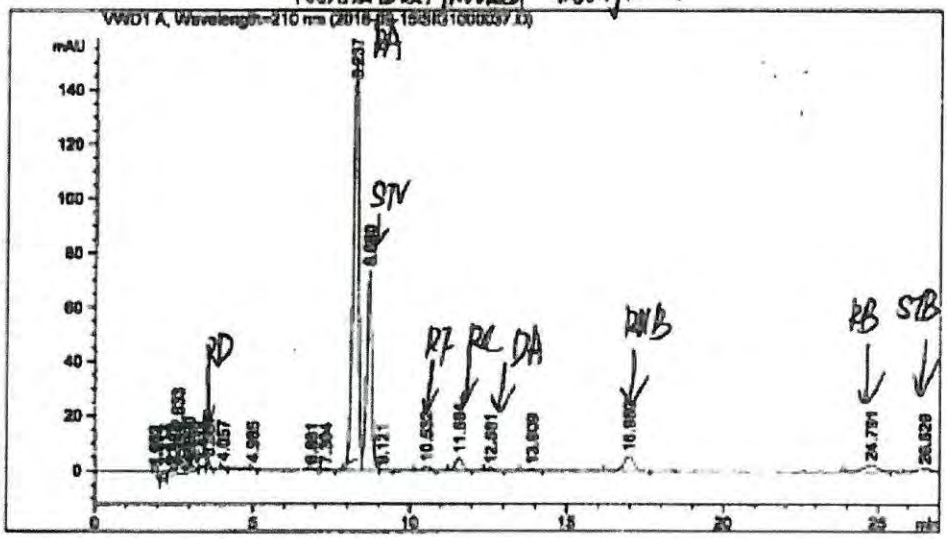
页 2/2

Batch No (b) (6)

data file
数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000037.D
样品名称:
Sample name

操作者 operator :
仪器 Instrument : 仪器 1
进样日期 Sample date 2016-9-16 3:27:02
position Sample bottle
位置 : 样品瓶 37
Sample size
进样量 : 手动 manual

collecting methods 采集方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2016-9-16 3:26:21
(调用后修改) invoked modification
analyse methods 分析方法 : D:\CHEM32\1\METHODS\TIANJUTANG.M
last modified 最后修改 : 2017-2-3 17:31:16
(调用后修改) invoked modification



面积百分比报告

排序 sort
信号 signal
乘积因子: Product factor : 1.0000
稀释因子: dilut ing factor : 1.0000
内标使用乘积因子和稀释因子
internal standard use product factor and diluting factor.

Signal 信号 1: VWD1 A, Wavelength=210 nm

Peak 峰 #	retention time 保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	1.902	BV	0.0770	9.51342	1.73355	0.2822
2	1.974	VB	0.1109	20.35961	2.86688	0.6040
3	2.213	BB	0.1493	28.51022	3.22033	0.8459
4	2.442	BV	0.1401	29.03539	2.68714	0.8614

仪器 1 2017-2-3 15:10:24 页 1/2
instrument

Batch No: (b) (6)

数据文件: D:\CHEM32\1\DATA\2016-09-15\SIG1000037.D

峰 #	retention time 保留时间 [min]	type 类型	Peak width 峰宽 [min]	Peak area 峰面积 [mAU*s]	Peak height 峰高 [mAU]	Peak area 峰面积 %
5	2.522	VV	0.0667	11.08694	2.40801	0.3289
6	2.633	VV	0.0712	75.04688	15.03273	2.2264
7	2.877	VV	0.0821	7.25547	1.32102	0.2152
8	2.990	VV	0.0828	19.32215	3.27139	0.5732
9	3.118	VB	0.1117	8.59491	1.13086	0.2550
10	3.414	BV	0.1145	8.71547	1.21702	0.2586
11	3.587	VB	0.0964	18.16843	2.88707	0.5390
12	4.057	VV	0.1379	11.22991	1.19216	0.3331
13	4.985	BV	0.1120	6.81364	9.69172e-1	0.2021
14	6.861	VB	0.1378	5.42353	5.76244e-1	0.1609
15	7.304	BB	0.1840	5.28982	4.40779e-1	0.1569
16	8.237	BV	0.1940	1870.68525	151.22502	55.7394
17	8.680	VV	0.1850	874.87805	73.43280	25.9543
18	9.121	VB	0.1829	5.16579	4.24783e-1	0.1532
19	10.532	VB	0.2278	22.75055	1.54519	0.6749
20	11.584	VB	0.2542	74.81031	4.58884	2.2193
21	12.581	VB	0.2715	14.37452	8.24164e-1	0.4264
22	13.909	VB	0.2907	11.15376	6.11851e-1	0.3309
23	16.980	VB	0.3515	120.18767	5.32127	3.5655
24	24.791	VV	0.4870	71.46282	2.22344	2.1200
25	26.529	BBA	0.4086	32.80493	1.03270	0.9732
总量 :				3370.83943	282.18441	

*** 报告结束 ***

RA = 62.64%
RD = 0.69%
STV = 23.65%
RT = 0.62%
RC = 2.02%
DA = 0.39%
RMB = 3.18%
RB = 1.89%
STB = 0.88%

仪器 1 2017-2-3 15:10:24

Appendix 4 Pesticide Testing Report for SXY Stevia® Total Steviol Glycosides 95%

eurofins

Analytical Report

Sample Code	**502-2016-00050203**	Report date	15-Sep-2016
Certificate No.	**AR-16-SU-047898-01-EN**		

SHANDONG SHENGXIANGYUAN BIOTECHNOLOGY CO.,LTD
Baojuan Peng
East of Chuangye Road/South of North Fangzhi Road
Qufu
Qufu

Our reference:	502-2016-00050203/ AR-16-SU-047898-01-EN
Client Sample Code:	20160902、20160903、20160904、20160905、20160906混合批
Sample described as:	stevia
Sample Packaging:	Sealed plastic bag
Sample reception date:	09-Sep-2016
Analysis starting date:	09-Sep-2016
Analysis ending date:	14-Sep-2016
Arrival Temperature (°C)	29.6
Sample Weight	380g
Sample Type	Powder

Residues		Results	Unit	LOQ	LOD
SU311	Pesticides Quechers Method: EN 15662:2008				
	Screened pesticides	Not Detected	mg/kg		
SU312	Pesticides Quechers Method: EN 15662:2008				
	Screened pesticides	Not Detected	mg/kg		

List of screened and not detected molecules (* = limit of quantification)

SU311 Pesticides Quechers (LOQ* mg/kg)

(a) 2-Phenylphenol (0.01)	(a) Acetochlor (0.01)	(a) Aclonifen (0.01)	(a) Aldrin (0.01)	(a) Ametryne (0.01)	(a) Anthraquinone (0.01)
(a) Aramite (0.04)	(a) Atrazine (0.01)	(a) Benfluralin (0.01)	(a) Bifenox (0.05)	(a) Bifenthrin (0.01)	(a) Biphenyl (0.01)
(a) Bromfenvinfos (0.01)	(a) Bromophos (0.01)	(a) Bromophos-ethyl (0.01)	(a) Bromopropylate (0.01)	(a) Butachlor (0.01)	(a) Butafenacil (0.01)
(a) Cadusafos (0.01)	(a) Captafol (0.05)	(a) Captan (0.01)	(a) Carbophenothion (0.01)	(a) Carbophenothion-methyl (0.01)	(a) Carboxin (0.01)
(a) Chlorbenside (0.01)	(a) Chlordane (Sum) ()	(a) Chlordane, alpha (0.01)	(a) Chlordane, gamma (0.01)	(a) Chlorfenapyr (0.01)	(a) Chlorfenson (0.02)
(a) Chlorfenvinphos (0.01)	(a) Chlormephos (0.02)	(a) Chloroneb (0.01)	(a) Chloropropylate (0.01)	(a) Chlorothalonil (0.01)	(a) Chlorothalonil (0.01)
(a) Chlorpyrifos (-ethyl) (0.01)	(a) Chlorpyrifos-methyl (0.01)	(a) Chlorthal-dimethyl (0.01)	(a) Chlorthion (0.02)	(a) Chlozolinate (0.01)	(a) Cinfumate (0.02)
(a) Cyanazine (0.02)	(a) Cyanofenphos (0.01)	(a) Cyanophos (0.02)	(a) Cyfluthrin (0.02)	(a) Cyhalothrin lambda- (0.01)	(a) Cypermethrin (0.02)
(a) Cyphenothrin (0.01)	(a) DDD, o,p'- (0.01)	(a) DDD, p,p'- (0.01)	(a) DDE, o,p'- (0.01)	(a) DDE, p,p'- (0.01)	(a) DDT (Sum) ()
(a) DDT, o,p'- (0.01)	(a) DDT, p,p'- (0.01)	(a) Deltamethrin (0.02)	(a) Dichlobenil (0.02)	(a) Dichlofenthion (3.01)	(a) Dichlofluanid (0.01)
(a) Dicloran (0.01)	(a) Dichlorobenzophenone o,p' (0.01)	(a) Dichlorobenzophenone p,p' (0.01)	(a) Dichlorvos (3.01)	(a) Dicofol (Sum) ()	(a) Dicofol, o,p'- (0.01)
(a) Dicofol, p,p'- (0.01)	(a) Dieldrin (0.01)	(a) Dieldrin (Sum) ()	(a) Dienochlor (0.02)	(a) Dinobuton (0.02)	(a) Dioxabenzofos (0.02)
(a) Dioxathion (0.02)	(a) Diphenylamine (0.01)	(a) Edifenphos (0.01)	(a) Endosulfan (Sum) ()	(a) Endosulfan, alpha- (0.01)	(a) Endosulfan, beta- (0.01)
(a) Endosulfan, sulfat- (0.01)	(a) Endrin (0.01)	(a) EPN (0.02)	(a) Ethalfluralin (0.01)	(a) Ethion (0.01)	(a) Etridiazole (0.01)
(a) Etrimfos (0.01)	(a) Fenarimol (0.01)	(a) Fenamiphos (0.01)	(a) Fenchlorphos (0.01)	(a) Fenchlorphos (sum) ()	(a) Fenchlorphos oxon (0.01)
(a) Fenitrothion (0.01)	(a) Fenpropathrin (0.01)	(a) Fenpropimorph (0.01)	(a) Fenson (0.01)	(a) Fenthion (0.01)	(a) Fenvalerate & Esfenvalerate (Sum of RSASR isomers) (0.01)
(a) Fenvalerate & Esfenvalerate(sum of RR,SS,RS,SR) ()	(a) Fenvalerate & Esfenvalerate(Sum of RR,SS isomers) (3.01)	(a) Fluchloralin (0.02)	(a) Flucythrinate (0.01)	(a) Flumetralin (3.02)	(a) Flumioxazin (0.01)
(a) Fluotrimazole (0.01)	(a) Fluquinconazole (3.01)	(a) Flusilazole-tau (0.01)	(a) Folpet (0.01)	(a) Folpet (Sum) ()	(a) Fonofos (0.01)
(a) Formothion (0.02)	(a) Halfenprox (0.01)	(a) HCB (0.01)	(a) HCH (Sum, without Lindan) ()	(a) HCH gamma(Lindan) (3.01)	(a) HCH, alpha- (0.01)
(a) HCH, beta- (0.01)	(a) HCH, delta- (0.01)	(a) HCH, epsilon- (0.01)	(a) Heptachlor (0.01)	(a) Heptachlor (Sum) ()	(a) Heptachlor epoxide cis (0.01)
(a) Heptachlor epoxide trans (0.01)	(a) Heptenophos (0.01)	(a) Iprobenfos (0.01)	(a) Isazofos (0.01)	(a) Isocarbophos (0.01)	(a) Isodrin (0.01)
(a) Isofenphos (0.01)	(a) Isofenphos-methyl (0.01)	(a) Isoprothiolane (0.01)	(a) Jodfenphos (0.01)	(a) Kresoxim-methyl (0.01)	(a) Lindrin (0.01)
(a) Malaoxon (0.01)	(a) Malathion (Sum) ()	(a) Mecarbam (0.01)	(a) Mepronil (0.02)	(a) Methacrifos (0.01)	(a) Methamidophos (0.1)
(a) Methidathion (0.01)	(a) Methoxychlor (0.01)	(a) Methyl Pentachlorophenylsulfide (0.01)	(a) Metribuzin (0.01)	(a) Mevinphos (0.01)	(a) Mirex (0.01)
(a) N-Desethyl-pirimiphos-methyl (0.01)	(a) Nitrapyrin (0.01)	(a) Nitrofen (0.01)	(a) Nitrothal-isopropyl (0.01)	(a) Octachlorodipropyl ether (0.421) (0.01)	(a) Ofurace (0.01)
(a) Oxadiazon (0.01)	(a) Oxychlordane (0.01)	(a) Oxyfluorfen (0.01)	(a) Paclobutrazol (0.01)	(a) Parathion (0.02)	(a) Parathion-methyl (0.02)
(a) PCB 101 (0.01)	(a) PCB 118 (0.01)	(a) PCB 138 (0.01)	(a) PCB 153 (0.01)	(a) PCB 180 (0.01)	(a) PCB 28 (0.01)
(a) PCB 52 (0.01)	(a) Pentachloroaniline (0.01)	(a) Pentachlorobenzole (0.01)	(a) Pentachlorobenzene (0.01)	(a) Permethrin (0.01)	(a) Phenkapton (0.02)
(a) Phenothrin (0.01)	(a) Phenthoate (0.01)	(a) Phorate (0.01)	(a) Phosphamidon (0.01)	(a) Phtalimid (0.01)	(a) Piperophos (0.01)
(a) Pirimiphos-ethyl (0.01)	(a) Pirimiphos-methyl (0.01)	(a) Procymidone (0.01)	(a) Profenofos (0.01)	(a) Profluralin (0.01)	(a) Prometryn (0.01)
(a) Propanil (0.01)	(a) Propazine (0.01)	(a) Prothiofos (0.01)	(a) Pyrazophos (0.01)	(a) Pyridaphenthion (0.01)	(a) Pyrifenox (0.01)
(a) Pyrifenox (0.01)	(a) Pyrimethanil (0.01)	(a) Quinalphos (0.01)	(a) Quintozene (0.01)	(a) Quizalofop-P-ethyl (0.01)	(a) Silafluofen (0.01)
(a) Silthiofam (0.01)	(a) Tebufenpyrad (0.01)	(a) Tecnazene (0.01)	(a) Teflubenzuron (0.01)	(a) Terbufos (0.01)	(a) Tetrachlorvinphos (0.01)

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SU311 Pesticides Quechers (LOQ* mg/kg)

(a) Tebufilon (0.01)	(a) Tetramethrin (0.01)	(a) Tecnazul (0.01)	(a) Tolylfluanid (0.01)	(a) Triallate (0.01)	(a) Triazamate (0.01)
(a) Triazophos (0.01)	(a) Trifloxystrobin (0.01)	(a) Trifluralin (0.01)	(a) Triflumizole (0.01)	(a) Uniconazole (0.02)	(a) Vinclozolin (0.01)

SU312 Pesticides Quechers (LOQ* mg/kg)

(a) 2,4-D (0.01)	(a) 2,4-D, total (0.01)	(a) 2,4-Formoxylidid (0.01)	(a) 3,4,5-Trimethacarb (0.01)	(a) 3-Hydroxycarbofuran (0.01)	(a) 4-CPA (0.01)
(a) Abamectin (Sum) ()	(a) Acephate (0.01)	(a) Acetamiprid (0.01)	(a) Azibenzolar-s-methyl (0.01)	(a) Azitluorfen (0.01)	(a) Azithiotrin (0.01)
(a) Alachlor (0.01)	(a) Aldicarb (0.01)	(a) Aldicarb (Sum) ()	(a) Aldicarb-sulfone (0.01)	(a) Aldicarb-sulfoxide (0.01)	(a) Amitraz (sum) ()
(a) Amitraz (0.01)	(a) Asulam (0.01)	(a) Avermectin B1a (0.01)	(a) Avermectin B1b (0.01)	(a) Azimsulfuron (0.01)	(a) Azinphos-ethyl (0.01)
(a) Azinphos-methyl (0.01)	(a) Azoxystrobin (0.01)	(a) Benalaxyl (0.01)	(a) Bendiocarb (0.01)	(a) Benoxacor (0.01)	(a) Bensulfuron-methyl (0.01)
(a) Bentazone (0.01)	(a) Bitertanol (0.01)	(a) Boscalid (0.01)	(a) Bromoxynil (0.01)	(a) Bromuconazole (Sum) ()	(a) Bromuconazole, cis- (0.01)
(a) Bromuconazole, trans- (0.01)	(a) Bupirimate (0.01)	(a) Buprofezin (0.01)	(a) Butocarboxim (0.05)	(a) Butoxycarboxim (Sum) ()	(a) Butoxycarboxim-sulfoxide (0.01)
(a) Butoxycarboxim (0.01)	(a) Carbaryl (0.01)	(a) Carbendazim/Benomyl (sum) (0.005)	(a) Carbofuran (0.01)	(a) Carbofuran (Sum) ()	(a) Carbosulfan (0.01)
(a) Carfentrazone-ethyl (0.01)	(a) Chloranthraniliprole (0.01)	(a) Chlorfluazuron (0.01)	(a) Chlorobenzuron (0.01)	(a) Chlorpropham (0.01)	(a) Chlorpyrifos (-ethyl) (0.01)
(a) Chlorpyrifos-methyl (0.01)	(a) Chromafenozide (0.01)	(a) Clethodim (0.01)	(a) Clofentazine (0.01)	(a) Clomazone (0.01)	(a) Clothianidin (0.01)
(a) Cyazofamid (0.01)	(a) Cymoxanil (0.01)	(a) Cyproconazole (0.01)	(a) Cyprodinil (0.01)	(a) Cyromazine (0.01)	(a) Demeton-S-methyl (0.01)
(a) Demeton-S-methyl-sulfone (0.01)	(a) Diazinon (0.01)	(a) Diethofencarb (0.01)	(a) Diethyl-m-toluamid (DEET) (0.01)	(a) Difenoconazole (0.01)	(a) Diflubenzuron (0.01)
(a) Diflufenican (0.01)	(a) Dimepiperate (0.01)	(a) Dimethachlor (0.01)	(a) Dimethoate (0.01)	(a) Dimethoate/Omethoate (sum) ()	(a) Dimethomorph (0.01)
(a) Diniconazole (0.01)	(a) Dinocap (0.01)	(a) Disulfoton (0.05)	(a) Disulfoton sulfoxide (0.01)	(a) Disulfoton-PS-sulfone (0.01)	(a) Diuron (0.01)
(a) Emamectin (Sum) ()	(a) Emamectin B1a (0.01)	(a) Emamectin B1b (0.01)	(a) Epoxiconazole (0.01)	(a) Ethiofencarb (0.01)	(a) Ethiofencarb (Sum) ()
(a) Ethiofencarb-sulfone (0.01)	(a) Ethiofencarb-sulfoxide (0.01)	(a) Ethoprophos (0.01)	(a) Etofenprox (0.01)	(a) Ethoxyquin (0.01)	(a) Fenarimol (0.01)
(a) Fenazaquin (0.01)	(a) Fenbuconazole (0.01)	(a) Fenhexamid (0.01)	(a) Fenobucarb (0.01)	(a) Fenoxycarb (0.01)	(a) Fenpropimorph (0.01)
(a) Fenpyroximate (0.01)	(a) Fensulfothion (0.01)	(a) Fensulfothion-oxon-sulfone (0.01)	(a) Fensulfothion-oxon-sulfoxide (0.01)	(a) Fensulfothion-PS-sulfone (0.01)	(a) Fenthion (0.01)
(a) Fenthion (sum) ()	(a) Fenthion-oxon (0.01)	(a) Fenthion-oxon-sulfone (0.01)	(a) Fenthion-oxon-sulfoxide (0.01)	(a) Fenthion-PS-sulfoxide (0.01)	(a) Fenthion-sulfone (0.01)
(a) Fipronil (0.005)	(a) Fipronil-sulfide (0.01)	(a) Fipronil-sulfone (0.01)	(a) Fluazifop-P-butyl (0.01)	(a) Fluazinam (0.01)	(a) Fludioxonil (0.01)
(a) Flufenoxuron (0.01)	(a) Fluopicolide (0.01)	(a) Fluotrimazole (0.01)	(a) Flutolanil (0.01)	(a) FM-6-1 (0.01)	(a) Fenoxanil (0.01)
(a) Forchlorfenuron (0.01)	(a) Formetanate (0.01)	(a) Furathiocarb (0.01)	(a) Hexaconazole (0.01)	(a) Hexaconazole (0.01)	(a) Hexaflumuron (0.01)
(a) Hexythiazox (0.01)	(a) Imazalil (0.01)	(a) Imibenconazole (0.01)	(a) Imidacloprid (0.01)	(a) Indoxacarb (0.01)	(a) Iodosulfuron-methyl (0.01)
(a) Iprodione (0.01)	(a) Iprovalicarb (0.01)	(a) Isoprocarb (0.01)	(a) Isoproturon (0.01)	(a) Linuron (0.01)	(a) Lufenuron (0.01)
(a) Malathion (0.01)	(a) Malathion (Sum) ()	(a) Mepanipyrim (0.01)	(a) Metalaxyl (0.01)	(a) Metamitron (0.01)	(a) Methamidophos (0.01)
(a) Methidathion (0.01)	(a) Methiocarb (0.01)	(a) Methiocarb (Sum) ()	(a) Methiocarb-sulfone (0.01)	(a) Methiocarb sulfoxide (0.01)	(a) Methomyl (0.01)
(a) Methomyl/Thiodicarb (sum) ()	(a) Methoxyfenozide (0.01)	(a) Metolachlor (0.01)	(a) Metoloarb (0.01)	(a) Monocrotophos (0.01)	(a) Myclobutanil (0.01)
(a) Napropamide (0.01)	(a) Neburon (0.01)	(a) Nicosulfuron (0.01)	(a) Nitenpyram (0.01)	(a) Novaluron (0.01)	(a) Nuarimol (0.01)
(a) Omethoate (0.01)	(a) Oxadixyl (0.01)	(a) Oxamyl (0.01)	(a) Oxamyl-oxime (0.01)	(a) Oxydemeton-methyl (0.01)	(a) Oxydemeton-methyl (sum) ()
(a) Paraoxon (0.01)	(a) Paraoxon-methyl (0.01)	(a) Penconazole (0.01)	(a) Pencycuron (0.01)	(a) Pendimethalin (0.01)	(a) Phorate (Sum) ()
(a) Phorate Sulfoxide (0.01)	(a) Phorate-sulfone (0.01)	(a) Phosalone (0.01)	(a) Phosmet (0.01)	(a) Phoxim (0.01)	(a) Piperonyl butoxide (0.01)
(a) Pirimicarb (0.01)	(a) Pirimicarb (Sum) ()	(a) Pirimicarb-desmethyl (0.01)	(a) Pirimicarb-Desmethylformamido (0.01)	(a) Pirimiphos-methyl (0.01)	(a) Primisulfuron-Methyl (0.01)
(a) Prochloraz (0.01)	(a) Procymidone (0.01)	(a) Promecarb (0.01)	(a) Propamocarb (0.01)	(a) Propargite (0.01)	(a) Propham (0.01)
(a) Propiconazole (0.01)	(a) Propoxur (0.01)	(a) Propoxycarbazone (0.01)	(a) Propyzamide (0.01)	(a) Prosulfocarb (0.01)	(a) Prosulfuron (0.01)
(a) Pymetrozine (0.01)	(a) Pyraclostrobin (0.01)	(a) Chlordecon (0.01)	(a) Pyrethrins (0.01)	(a) Pyridaben (0.01)	(a) Pyrimethanil (0.01)
(a) Pyriproxyfen (0.01)	(a) Quinoxyfen (0.01)	(a) Resmethrin (0.01)	(a) Rimsulfuron (0.01)	(a) Rotenone (0.01)	(a) Sethoxydim (0.01)
(a) Simazine (0.01)	(a) Simeconazole (0.01)	(a) Spirmesd (0.01)	(a) Spinosyn A (0.01)	(a) Spinosyn D (0.01)	(a) Spirodiclofen (0.01)
(a) Spiromesifen (0.01)	(a) Spirotetramine (0.01)	(a) Tebuconazole (0.01)	(a) Tebufenozide (0.01)	(a) Tebufenpyrad (0.01)	(a) Teflubenzuron (0.01)
(a) Tepraloxydim (0.01)	(a) Tetraconazole (0.01)	(a) Thiabendazole (0.01)	(a) Thiacloprid (0.01)	(a) Thiamethoxam (0.01)	(a) Thiamethoxam (Sum) ()
(a) Thifensulfuron-methyl (0.01)	(a) Thiodicarb (0.01)	(a) Thiofanox sulfone (0.01)	(a) Thiofanox sulfoxide (0.01)	(a) Thiophanate-methyl (0.01)	(a) Tolclofos-methyl (0.01)
(a) Tralkoxydim (0.01)	(a) Triadimefon (0.01)	(a) Triadimefon and triadimenol ()	(a) Triadimenol (0.01)	(a) Triasulfuron (0.01)	(a) Triasulfuron methyl (0.01)
(a) Tribenuron-methyl (0.01)	(a) Trichlorfon (0.01)	(a) Tridemorph (0.01)	(a) Trifloxystrobin (0.01)	(a) Trifloxysulfuron (0.01)	(a) Triflumizol/FM-6-1 (Sum) ()
(a) Triflumizole (0.01)	(a) Triflumuron (0.01)	(a) Trifusulfuron-methyl (0.01)	(a) Vamidothion (0.01)	(a) Vamidothion-sulfone (0.01)	(a) Vamidothion-sulfoxide (0.01)
(a) Zoxamide (0.01)					

SIGNATURE (b) (6)

Pathik Vyas
Technical Director

EXPLANATORY NOTE
≥ Greater than or equal to
< Less than
≤ Less than or equal to
N/A means Not applicable
Not Detected means not detected at or above the Limit of Quantification (LOQ)
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group
The result(s) relate(s) only to the item (s) tested.
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END OF REPORT

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检测报告

实验室样品编号	**502-2016-00050203**	报告日期	15-Sep-2016
报告编号	**AR-16-SU-047898-01-ZH**		

山东圣香远生物科技有限公司
彭保娟
山东省曲阜市纺织北路南创业大道东
Qufu

样品编号：	502-2016-00050203/ AR-16-SU-047898-01-ZH		
客户样品编号：	20160902、20160903、20160904、20160905、20160906混合批		
样品描述：	甜菊糖苷		
样品包装：	密封塑料袋		
样品接收日期：	09-Sep-2016		
检测开始日期：	09-Sep-2016		
检测结束日期：	14-Sep-2016		
接收时样品温度 (°C)	29.6	样品重量	380g
样品类型	干粉		

残留物	结果	单位	定量限	检出限
SU311 Quechers GC/MS检测农药列表 方法：Quechers, EN 15662				
所有扫描的农药	未检出	mg/kg		
SU312 Quechers LC-MS/MS检测农药列表 方法：Quechers, EN 15662				
所有扫描的农药	未检出	mg/kg		

完整的参数列表 (* = 定量限)

SU311 Quechers GC/MS检测农药列表 (LOQ* mg/kg)

[illegible]

SU312 Quechers LC-MS/MS检测农药列表 (LOQ* mg/kg)

[illegible]

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页 2/2
AR-16-SU-047898-01-ZH

SU312 Quechers LC-MS/MS检测农药列表 (LOQ* mg/kg)

(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] B1a (0.01)	(a) [illegible] B1b (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] ([illegible]) ()	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 丁[illegible] (0.05)	(a) 丁[illegible] [illegible] ()	(a) 丁[illegible] (0.01)
(a) 丁[illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.005)	(a) [illegible] (0.01)	(a) [illegible] ([illegible]) ()	(a) 丁[illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] ([illegible]) (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) 二[illegible] (0.01)	(a) 乙[illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 二[illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] ([illegible]) ()	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 乙[illegible] (0.05)	(a) 乙[illegible] (0.01)	(a) 乙[illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] [illegible] ()	(a) [illegible] B1a (0.01)	(a) [illegible] B1b (0.01)	(a) [illegible] (0.01)	(a) 乙[illegible] (0.01)	(a) 乙[illegible] [illegible] ()
(a) 乙[illegible] (0.01)	(a) 乙[illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 乙[illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 丁[illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] ([illegible]) ()	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.005)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 4-[illegible]-o,o,p-三[illegible]-N-(1-[illegible]-2-[illegible] [illegible]乙基)-o-[illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 己[illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] [illegible] ()	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] [illegible] ()	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible]([illegible]) ()	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible]([illegible]) ()
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) 二[illegible] (0.01)	(a) [illegible] [illegible] ()
(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)	(a) [illegible] (0.01)
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Pathik Vyas
技术总监

注释

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Appendix 5 Estimated Daily Intake Levels of SXY Stevia® Total Steviol Glycosides 95%

There have been continuing studies to estimate the intake of steviol glycosides. Most recently, Dewinter et al. (2016) investigated the dietary intake of non-nutritive sweeteners, including steviol glycosides, in children with type 1 diabetes. Using a phased tier approach, the tier 2 (maximum concentration) and tier 3 (maximum used concentrations) exposures were assessed based on survey data obtained from patients at the Pediatrics Department of the University Hospitals Leuven (Belgium). In both tier 2 and tier 3 exposure assessments, high consumers (P95) aged 4-6 years old were estimated to have a steviol glycosides intake higher than the ADI, calculated at 119% of ADI. The authors noted that the exposure assessment is a worst-case scenario since "it is assumed that all processed foods in which the food additive is authorized contain the food additive at the [maximum permitted levels]." Furthermore, Dewinter et al. conclude that there is little chance that children with type 1 diabetes will exceed ADIs for steviol glycosides.

A. Food Uses as Addressed by JECFA, Merisant & Cargill

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 5-1. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 5-2). Merisant utilized food consumption survey data from 2003-2004 National Health and Nutrition Examination Survey (NHANES) to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90th precentile daily consumption levels of rebaudioside A were estimated as 2.0 and 4.7 mg per kg bw per day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that, with a few minor exceptions, rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates and the intake estimates are presented in Table 5-3.

B. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 5-1 were utilized to gauge the potential human exposures of rebaudioside A and steviol glycosides and in foods as reported in the US and in other countries. As rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly.

Table 5-1. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

Food Type	Maximum Use Level Reported[a] (mg steviol glycosides /kg of food)	Maximum Use Level Calculated for rebaudioside A[b] mg rebaudioside A /kg of food	Maximum Use Level Calculated for rebaudioside A[b] mg steviol equivalents /kg of food
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

[a] Reproduced from WHO (2006).

[b] Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

Table 5-2. Proposed Uses & Levels of Rebaudioside A by Merisant[a]

Food Uses	Reb A (ppm)
Tabletop sweeteners	30,000[b]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Merisant (2008)

[b] Reb A content of sachet prior to dilution and not representative of "as consumed."

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described below, the multiple approaches tended to converge to yield estimated daily intakes

(EDIs) in the range of 1.3 – 4.7 mg per kg bw per day that, when compared to the acceptable daily intake (ADI), constitutes supporting information in the subject GRAS evaluation.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg per kg bw per day with the African diet to 3.5 mg per kg bw per day with the European diet. Additionally, JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 5-4.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg per kg bw per day on a steviol basis or 3.0 – 4.5 mg per kg bw per day for rebaudioside A based on the molecular weight adjustment. Similarly, FSANZ (2008) estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario, which resulted in estimated exposures of 0.3 - 1.0 mg per kg bw per day for the mean and 90th percentile consumer, or 0.5 – 1.5 mg per kg bw per day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. FSANZ examined consumption in other age groups and concluded that there were no safety concerns for children of any age. Merisant also calculated a dietary estimate for Reb A of 2.0 mg per kg bw per day for the average consumer and 4.7 mg per kg bw per day for a 90th percentile consumer. On a steviol equivalent basis, the Merisant estimates would be 0.7 and 1.6 mg per kg bw per day, respectively. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg per kg bw per day when calculated as Reb A (Renwick, 2008).

Table 5-3. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of EDI

Scenarios	EDI		
	As Steviol[a] (mg/kg bw/day)	As Rebaudioside A[b] (mg/kg bw/day)	Total Daily Intake[c] (mg/day)
JECFA			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
FSANZ			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
MERISANT			
		2.0 - 4.7[d]	120 - 282
CARGILL			
		1.3 - 3.4[d]	78 - 204

[a] Published values for mixed steviol glycosides consumption listed in this column were used for the calculation of Reb A consumption values appearing in next two columns.

[b] Estimates for Reb A consumption were calculated from JECFA and FSANZ estimates as steviol by multiplying by 3 to correct for the molecular weight of Reb A compared to steviol and by subsequently dividing by 2 because of the increased inherent sweetness of Reb A compared to the mixed steviol glycosides.

[c] Total daily intake figures were calculated for a 60 kg adult.

[d] Published values are shown for comparison purposes.

Table 5-4. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

Estimate	Exposure (mg/kg bw/day)
GEMS/Food (International)[a]	1.3 -3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.

[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

In October 2009, Cargill applied to FSANZ to increase the maximum usage levels of high purity steviol glycosides in the high-volume food categories of ice cream and various beverages. Cargill supported its application with increased usage levels by presenting market share analyses that overestimate actual intake while remaining well below the generally accepted ADI. In December 2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved the Cargill application to increase the allowed maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).

On January 13, 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent). For European children aged 1-14, revised intake estimates ranged from 1.7 to 16.3 mg per kg bw per day, and for adults, the range was reported to be from 5.6 to 6.8 mg per kg bw per day (EFSA, 2011b).

Most recently, Roberts et al. (2016) suggested that a higher ADI is justified based on metabolic factors to reduce the 100X safety factor. A chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, was determined by comparative studies in rats and humans. A CSAF that is less than the standard 100X safety factor will result in an increase in the ADI, independent of the NOAEL. The authors determined that using a CSAF can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides, depending on whether area under the plasma-concentration time curve (AUC) or C_{max} data are used when considering the 1,000 mg per kg bw per day NOAEL (which is equivalent to 400 mg per kg bw per day of steviol) for stevioside reported by Toyoda et al. (1997).

There have been many scholarly estimates of potential dietary intake of replacement sweeteners---including steviol glycosides---that have been published (FSANZ, 2008, Renwick, 2008, WHO, 2003) or submitted to FDA (Merisant, 2008). In GRN 301, a simplified estimate was proposed to and accepted by FDA based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg per kg bw per day for any population subgroup.

Appendix 6 Summary of Published Safety Reviews

1. Summary of JECFA Reviews

At an early review during its 51st meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.
>
> In view of the absence of information for the elaboration of specifications for stevioside and since the evaluation of the available toxicological data revealed several limitations, the Committee was unable to relate the results of the toxicological investigations to the commercial product and could not allocate an ADI to stevioside.
>
> Before reviewing stevioside again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested that the common steviol glycosides are converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with new toxicology studies on test materials that consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The new information also revealed that in *in vitro* studies, steviol is mutagenic, while in *in vivo* conditions, it is not mutagenic. The committee became convinced that purified steviol glycosides did not impair reproductive performance, as did crude preparations of stevia, and that there were sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable ADI in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are

not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.

The NOEL for stevioside was 970 mg per kg bw per day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg per kg bw per day (equivalent to 5–10 mg per kg bw per day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).

The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg per kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg per kg bw per day (or 383 mg per kg bw per day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.

The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68th meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol glycosides. The Chemical and Technical Assessment report, written after the 2007 meeting, explained the Committee's thinking, which resulted in flexibility in the identity specifications (FAO, 2007b, FAO, 2007a).

In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.

Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of

compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

In 2008, based on additional clinical studies, at its 69th meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008), raised the ADI to 0 – 4 mg per kg bw per day, and removed the "temporary" designation. The summary of the Committee's key conclusions in the final toxicology monograph addendum (WHO, 2009) were stated as follows:

> From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg per kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0–2 mg per kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg per kg bw per day (383 mg per kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg per kg bw per day (5–10 mg per kg bw per day expressed as steviol).
>
> The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg per kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg per kg bw expressed as steviol.
>
> The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

2. Summary of FSANZ Review of Steviol Glycosides

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. FSANZ concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose, or other parameters in normal, hypotensive, or diabetic subjects at doses up to 11 mg per kg bw per day. FSANZ agreed with JECFA in setting an ADI of 4 mg steviol equivalents per kg bw per day, which was derived by applying a 100-fold safety factor to the NOEL of 970 mg per kg bw per day established by a 2-year rat study (Toyoda et al., 1997). The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al. (2008), which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-

fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg per kg bw per day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970 mg per kg bw per day (equivalent to 383 mg per kg bw per day steviol) in a 2-year rat study (FSANZ, 2008). In December 2010, FSANZ recommended accepting the increased usage levels since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).

3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier---in 1984, 1989 and 1999---the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was "toxicologically not acceptable" due to insufficient available data to assess its safety. However, in light of JECFA's 2008 findings, and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener.

As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per kg bw per day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside per kg bw per day (corresponding to approximately 388 mg steviol equivalents per kg bw per day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

Recently, EFSA (2011b) revised its exposure assessment of steviol glycosides from its uses as a food additive for children and adults, and published the reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups. Additionally, 15 other foods were removed, mainly within the category of desserts and other products, while 3 new food uses were added. The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg per kg bw per day and from 1.7 to 16.3 mg per kg bw per day at the 95th percentile. A correction was considered to be necessary for the consumption of non-

alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95th percentile for children ranged from 1.0 to 12.7 mg per kg bw per day. For adults, the mean and 97.5th percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg per kg bw per day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent).

In addition, EFSA (2011a) recently accepted rebaudioside A as a flavoring agent in a variety of foods. EFSA reviewed the available safety data on rebaudioside A and agreed that the ADI of 4 mg per kg bw per day established for steviol glycosides applied also to rebaudioside A in a purified form. The dietary intake for use as a flavoring agent was calculated by two different methods, and EFSA determined that the worst-case exposure would be 10,888 microgram per person per day, which is equivalent to 181 microgram rebaudioside A per kg bw per day, for a person weighing 60 kg. This corresponds to a daily intake of 60 microgram steviol per kg bw per day, using a conversion factor of 0.33 for converting the amount of rebaudioside A into steviol equivalents.

4. Other Published Reviews

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008, Geuns, 2003, Huxtable, 2002). Four additional reviews have appeared on the toxicology and biological activity of stevia extracts and steviol glycosides (Yadav and Guleria, 2012, Brown and Rother, 2012, Brahmachari et al., 2011, Chatsudthipong and Muanprasat, 2009). In reviewing these studies, caution is warranted since these reviews do not differentiate well between studies on crude stevia extract and purified steivol glycosides. In addition, many of the reviewed studies on biological activity used routes of administration other than oral, and they may have used doses that are much higher than expected dietary exposures of steviol glycosides as a sweetener. In a letter to the editor of the Journal of Pharmacology and Therapeutics, Roberts and Munro (2009) criticized the Chatsudthipong and Muanprasat (2009) review with some important points that are applicable in general to these four reviews. Important excerpts from this letter are as follows:

> "It is well established that some stevia extracts are crude mixtures that contain multiple components of the stevia leaf, including those components that do not provide a sweet taste. These mixtures also vary considerably in quality, purity, and composition. Therefore, it is not surprising that sometimes these crude and uncharacterized materials may contain substances that possess some degree of pharmacologic activity but any such effects cannot be attributed specifically to the steviol glycosides. In contrast to studies conducted with less pure steviol glycoside preparations, studies conducted with purified preparations do not indicate any evidence of pharmacological effects."
>
> "The authors consistently cite pharmacological, toxicological, and biochemical effects from in vitro studies or from studies in which animals were dosed intravenously (e.g., Melis, 1992 a,b,c). Steviol glycosides are hydrolyzed completely by the gut microflora to steviolprior to absorption, with no systemic absorption of the glycone form following oral exposure. Therefore, the results of in vitro and

> intravenous, intraperitoneal, or subcutaneous dosing studies of the glycone form are not relevant to the safety of steviol glycosides consumed orally."
>
> "Collectively, the report of Chatsudthipong and Muanprasat (2009) is incomplete and lacking discussion of key studies of the safety of stevioside and rebaudioside A. It focuses on alleged effects of stevia and steviol glycosides of low or unknown purity, fails to consider the route of exposure in relation to metabolism and safety assessment and does not include recent opinions expressed by world wide regulatory authorities affirming the safety of purified forms of stevioside and rebaudioside A as a food ingredient."

Most recently, Urban et al. (2015) reviewed the potential allergenicity of steviol glycosides. The authors noted that: "hypersensitivity reactions to stevia in any form are rare" and concluded that current data do not support claims that steviol glycosides are allergenic. In addition, the authors stated that there is "little substantiated scientific evidence" to warrant consumer warning labels for highly purified stevia extracts (Urban et al., 2015).

Appendix 7 Studies on Steviol Glycosides Preparations That Are Primarily Mixtures of Stevioside & Rebaudioside A

This appendix summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. In some of the published literature, the terms stevia, stevioside, and stevia glycoside are used interchangeably. However, wherever possible, an attempt has been made to identify the specific substance studied.

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Several studies in rats (Wingard Jr et al., 1980, Nakayama et al., 1986, Koyama et al., 2003) and other animal models, including chickens (Geuns et al., 2003b), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003a), indicate that stevioside is not readily absorbed from the GI tract. Available evidence from *in vitro* metabolism studies suggests that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns, 2003, Koyama et al., 2003). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol following oral administration of steviosides to rats. In studies with human and rat liver extracts, Koyama et al. (2003) demonstrated that steviol can be converted to various glucuronides. Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999). Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003a).

Koyama et al. (2003) published an *in vitro* study in which α-glucosylated steviol glycosides were degraded by fecal microflora to steviol glycosides. These are subsequently hydrolyzed to the aglycone, steviol, demonstrating that the metabolic fate of α-glucosylated steviol glycosides follows that of non-modified steviol glycosides. Due to the similarities in metabolic fate, the safety of α-glucosylated steviol glycosides can be established based on studies conducted with non-modified steviol glycosides. Furthermore, as individual steviol glycosides show similar pharmacokinetics in the rat and humans, the results of toxicology studies on individual steviol glycosides are applicable to the safety of steviol glycosides in general.

In a human study with 10 healthy subjects, Geuns et al. (2006) measured blood, urine, and fecal metabolites in subjects that received 3 doses of 250 mg of purified stevioside (>97%) three times a day for 3 days. Urine was collected for 24 hours on day 3, and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine, and feces. Approximately 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on these measurements, the authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.

In a recent publication, Renwick and Tarka (2008) reviewed studies on microbial hydrolysis of steviol glycosides. The reviewers concluded that stevioside and Reb A are not absorbed directly, and both are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for Reb A than for stevioside. Studies have shown that steviol-16,17-epoxide is not a microbial metabolite.

2. Acute Toxicity Studies

The oral LD_{50} studies of stevioside (purity, 96%) following administration of a single dose to rodents are summarized in Table 7-1. No lethality was noted within 14 days after the administration, and no clinical signs of toxicity, or morphological or histopathological changes were found, indicating that stevioside is relatively harmless.

Table 7-1. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

Species	Sex	LD_{50} (g/kg bw)	Reference
Mouse	Male and Female	>15	Toskulkac et al. (1997)
Mouse	Male	> 2	Medon et al. (1982)
Rat	Male and Female	>15	Toskulkac et al. (1997)
Hamster	Male and Female	>15	Toskulkac et al. (1997)

3. Subchronic Toxicity Studies

In five published studies, subchronic toxicity of stevioside was investigated in rats following oral administration. In addition, a reproduction study in hamsters included subchronic phases on the F_0, F_1, and F_2 generations. These studies are summarized in Table 7-2. One of these studies was particularly important because it served as a range-finding study for two subsequent chronic studies. In this 13-week toxicity study, Fischer 344 rats (10 per sex per group) were given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1,300, and 2,500 mg per kg bw per day) to determine the appropriate doses for a two-year carcinogenicity study. None of the animals died during the administration period, and there was no difference in body-weight gain between the control and treated groups during administration or in food consumption in the latter part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific, because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1990).

In earlier 3-month rat studies reviewed by Geuns (2003)---the sample purity, doses, strain of rat were not reported---a no effect level was determined to be in excess of 2,500 mg per kg bw per

day and 7% of the diet, apparently due to lack of effects at the highest dose tested in both studies (Akashi and Yokoyama, 1975).

In a recently published exploratory subchronic toxicity study, Awney et al. (2011) investigated the effects of 97% pure stevioside on body weight, organ relative weight, hematological and biochemical parameters, and enzyme activities in Sprague Dawley rats. In this 12-week toxicity study, groups of male rats (8 per group) were given drinking water containing stevioside. The groups were assigned to drink distilled water (control), low-dose stevioside solution (15 mg per kg per day), high-dose stevioside solution (1,500 mg per kg per day), or low-dose stevioside (15 mg per kg per day) plus inulin solution for 12 weeks as the sole source of liquid. Fluid intake was recorded daily, and levels of test articles were adjusted weekly to receive the appropriate target concentration. Low-dose stevioside (15 mg per kg bw per day) administration, with or without inulin, for 12 weeks did not reveal any adverse effects on body weight, organs relative weight, hematological and biochemical parameters, or enzyme activities. However, treatment with high-dose stevioside was reported to cause significant changes in several investigated toxicological parameters. Among the hematological parameters, significant changes were noted in all except white blood cells (WBCs), red blood cells (RBCs), and packed cell volume (PCV%), and in all clinical chemistry parameters except proteins, total lipids, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These data support the NOEL of 15 mg per kg per day. However, critical review of the publication reveals that the study was poorly designed and implemented. Design deficiencies include: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water, resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection, which affects many chemistry and hematological values; no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. In addition to these study design deficiencies, the report fails to adequately present mean or individual organ weight data and, in general, there appears to be inadequate comparison of study findings against laboratory historical control data. Any one of these oversights could have adversely affected the results and/or interpretation of the hematological and chemistry data.

In addition to the above-described parameters, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased (Awney et al., 2011). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. This enzyme was not measured in any previous steviol glycosides studies nor has it been adequately vetted for application in toxicological studies. These investigators did not identify the specific TRAP isomer measured, the methodology employed, the handling of the samples, or any historical data on TRAP levels. The significance and relevance of this poorly documented toxicological endpoint, which lacks histopathological confirmation, does not appear to have a distinct role in determining the toxicological profile of a material in a test animal. The data presented by Awney et al. (2011) are probably not representative of changes due to the subchronic dietary administration of steviol glycosides because of overall inadequate study design and reliance on the findings of the untested enzyme TRAP. The preponderance of the data from

several well-designed studies on steviol glycosides suggest that differences noted in hematological and chemistry data are probably random, nonspecific, and not toxicologically significant.

Critical reviews of the publication by Carakostas (2012) and Waddell (2011) revealed a poor study design that included: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection, which affects many chemistry and hematological values; no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately describe mean or individual organ weight data and lacked comparison of study findings against laboratory historical control data.

Table 7-2. Summary of Subchronic Studies on Stevioside

Study	Animal Model/ Group size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Aze et al. (1990)[a]	F344 rat/ 10 females & 10 males in each of 6 groups	Stevioside/ Not reported	0, 0.31, 0.62, 1.25, 2.5, 5% in diet/13 weeks	Not reported	No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels & histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in clear dose-response relationship. Investigators did not consider these changes to be treatment related due to small magnitude & low severity of changes, the lack of clear dose relationship & limitation to males only. Organ weights, urine chemistry & gross necropsy not discussed. Authors concluded that 5% stevioside in diet is tolerable dose for 2 year study.
Mitsuhashi (1976)[b]	Rat (strain not reported)	Stevioside/ Not reported	Dietary concentrations up to 7%/ 3 months	Not reported	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy & histopathology not discussed.
Akashi and Yokoyama (1975)[b]	Rat (strain not reported)	Stevioside/ Not reported	Oral doses up to 2,500 mg/kg bw/3 months	2,500	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy & histopathology not discussed.
Awney et al. (2011)	Sprague Dawley rats	Stevioside 97%	Drinking water (15, 1,500 mg/kg bw /day)	15	Treatment with high dose stevioside caused significant changes in several investigated toxicological parameters. Among hematological parameters, significant changes noted in all except WBCs, RBCs& PCV% & in all clinical chemistry parameters except proteins, total lipids, ATL and AST.

[a] Abstract only. [b] As reported by Geuns (2003).

4. Chronic Toxicity Studies

Chronic effects of stevioside have been studied in three separate studies (Table 7-3). No treatment-related increase in tumor incidence was seen in any of these studies. In the most recent and well-documented study {additional study details were presented to JECFA in 2006 (WHO, 2006), the apparent no observed adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% [test sample purity 96%, Toyoda et al. (1997)]. At 5% of the diet, statistically significant decreases in body weight, percent survival, and kidney weight were noted. The authors attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The authors reported that this tumor is rather common in F344 rats and that the overall incidence in male rats was actually within the historical control range experienced in the laboratory where studies were conducted. The authors attributed the decrease in kidney weight as probably due to a decrease in chronic inflammation found in the histopathological examination relative to control animals.

Table 7-3. Summary of Chronic Toxicity Studies on Stevioside

Study	Animal Model/ Group Size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Toyoda et al. (1997)	F344 rat/ 50 per sex per group	95.6% Stevioside	*Ad libitum* 0,2.5, 5% of diet/~24 months (104 weeks)	Author did not assign a NOAEL. (Mid-dose calculates to 970 in males; JECFA, 2006)	Significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological & organ weights observed. Body weight gains dose-dependently decreased in both sexes. Kidney weights significantly lower in 5% males& ovary, kidney, & brain weights significantly increased in 5% females. Tumors& non-neoplastic lesions found in all groups& not correlated to treatment. Conclusion--stevioside is not carcinogenic under these experimental conditions.
Xili et al. (1992)[a]	Wistar rat/ 45 per sex per group	85% Stevioside	0, 0.2, 0.6, 1.2 % of diet/24 months	794 (high dose)	After 6, 12 & 24 months 5 rats from each group sacrificed for analysis. No effects observed on growth, food utilization, general appearance, mortality, or lifespan. No changes in hematological, urinary, or clinical biochemical values. Histopathological analysis showed that the neoplastic and non-neoplastic lesions unrelated to level of stevioside in diet.
Yamada et al. (1985)	F344 rat/ 70 per sex per group, 30 per sex per group in low-dose	95.2% Steviol glycosides (75% stevioside; 16% Reb A)	0.1, 0.3, 1% of diet/22 months for males, 24 months for females	550 (high dose)	At 6 &12 months, 10 males & 10 females sacrificed for analysis. General behavior, growth & mortality were same among groups throughout experiment. At 6 months, protein urea significantly increased in females, & blood glucose increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate & testes increased in males at 6 months, &weight of ovaries decreased in females in dose-dependent manner.

					Histopathological examination showed differences in various organs at 6 months that were unrelated to stevioside dose. These differences not found at 12 months. Authors concluded that there were no significant changes after 2 years.

[a] Only abstract available.

5. Reproductive & Developmental Toxicity Studies

The use of *S. rebaudiana* as an oral contraceptive has been reported by Indians in Paraguay (Planas and Kuć, 1968, Schvartaman et al., 1977). In experimental studies in rats, crude stevia leaf extract has been shown to inhibit fertility (Planas and Kuć, 1968). Reproductive toxicity studies have been conducted with orally administered purified stevioside. No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2,500 mg per kg per day (Yodyingyuad and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% [equivalent to 3,000 mg per kg bw per day; sample purity 96%; Mori et al. (1981)]. Similar results were observed in an additional rat study that was reviewed by Geuns (2003) where limited information is available in English (Usami et al., 1994).

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1,000 mg per kg bw per day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg per kg bw per day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

No effect on pregnancy or developmental parameters were observed in Swiss albino mice with stevioside or aqueous stevia extract at doses up to 800 mg per kg bw per day in female mice (Kumar and Oommen, 2008). Further details on these studies to the extent available are presented in Table 7-4.

Table 7-4. Summary of Reproductive Toxicity Studies on Steviol Glycosides

Study	Animal Model/ Group Size	Test Sample Purity Stevioside (Unless Otherwise Noted)	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results & Remarks
Kumar and Oommen (2008)	Swiss albino mice/ 4 groups of 5 females	Not reported	500 & 800 mg/kg bw/15 days	800	Stevioside & stevia extract (purity & composition not reported) did not have any effect on reproductive parameters in mice when administered to female mice before or during pregnancy. No changes seen in number of implantations or uterine resorptions. No gross anatomical or histopathologic effects seen in 16-day embryos.
Usami et al. (1994)[a]	Wistar Rat/4 groups of 25 or 26 pregnant rats	95.6%[b]	0, 250, 500, 1,000 mg/kg bw/10 days	1,000	Pregnant rats given doses of stevioside by gavage once/day on days 6-15 of gestation & were sacrificed on day 20 of gestation. Fetuses examined for malformations in addition to maternal & fetal body weight, number of live fetuses, sex distribution& numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside not teratogenic in rats.
Yodyingyuad and Bunyawong (1991)	Hamster/ 10 male, 10 female per group (40 total)	90%	0, 500, 1,000, 2,500 mg/kg bw/day/ duration unclear/ 3 months	2,500	Males from each group mated to females from respective dose group. Each female allowed to bear 3 litters during course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F_1 & F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth & fertility. Histological examination showed no effect on reproductive organs at any dose.
Oliveira-Filho et al. (1989)[a]	Rat/ number not reported	Not reported (Dried Stevia Leaves)	0 or 0.67 g dried leaves/mL, 2 mL twice per day/ 60 days	Not reported	Prepubertal rats (25-30 days old) tested for glycemia; serum concentrations of thyroxine; tri-iodothyroxine; available binding sites in thyroid hormone-binding proteins; binding of ^{3}H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, & pancreas; water content of testes & prostate; body-weight gain; & final weights of testes, prostate, seminal vesicle, submandibular salivary gland& adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control.
Mori et al. (1981)	Rat/11 male, 11 female per group (44 total)	96%	0, 0.15, 0.75 or 3 % of feed/60 days	2,000	Males given stevioside dose in diet for 60 days before & during mating with females who received same diet (as mated male) 14 days before mating & 7 days during gestation. No effect due to treatment on fertility or mating performance& no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead & resorbed fetuses at highest dose.

Planas and Kuć (1968)	Rat/14 per group (28 total)	Not reported (Crude stevia extract)	0 or 5% Crude stevia extract /18 days	Not reported	Extract given orally to adult female rats for 12 days, who were mated with untreated males during last 6 days. Fertility reduced to 21% of fertility in control rats & remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and & necropsy not discussed.

[a] Only abstract available. [b] As reported by EuropeanCommission (1999b).

6. Mutagenicity & Genotoxicity Studies

In a series of studies, mutagenic and genotoxic effects of various stevia extracts and various preparations of stevioside were investigated. These studies are summarized in Table 7-5. All studies were negative with the exception of a comet assay done in rats (Nunes et al., 2007a). The methodology used in this study, and the resulting conclusions, have been questioned by Geuns (2007b), Williams (2007), and Brusick (2008), and responded to by the authors (Nunes et al., 2007c, Nunes et al., 2007b).

In a recent review, Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two recent publications (Brahmachari et al., 2011, Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile (Urban et al., 2013). The review aimed to address this matter by evaluating the specific genotoxicity studies of concern, while evaluating the adequacy of the database that includes more recent genotoxicity data not noted in these publications. The results of this literature review showed that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust, and does not indicate that either stevioside or rebaudioside A are genotoxic. This finding, combined with lack of carcinogenic activity in several rat bioassays, establishes the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential.

Table 7-5. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
In Vitro						
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[a] 1 mg/plate[b]	Negative	Matsui et al. (1996)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[c]	Suttajit et al. (1993)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	NS	50 mg/plate	Negative	Klongpanichpak et al. (1997)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[c]	Matsui et al. (1996)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[c]	Pezzuto et al. (1985)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[c]	Medon et al. (1982)

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
Gene mutation	Mouse lymphoma L5178Y cells, TK- locus	Stevioside	NS	5 mg/mL	Negative[c,d]	Oh et al. (1999)
Gene mutation (umu)	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[c]	Matsui et al. (1996)
Gene mutation	*B. subtilis* H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative[c]	Matsui et al. (1996)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/mL 12 mg/mL	Negative	Matsui et al. (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/mL	Negative	Suttajit et al. (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/mL	Negative[a]	Ishidate et al. (1984)
In Vivo						
DNA damage (comet assay)	Wistar rats; liver, brain and spleen	Stevioside	88.62	4 mg/L (estimated to be 80 - 500 mg/kg bw/day) in drinking water for 45 days	Positive in all tissues examined, most notably in liver	Nunes et al. (2007a)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52; Reb A, 22	250 – 2,000 mg/kg bw	Negative[e]	Sekihashi et al. (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Stevia	NS	2,000 mg/kg bw	Negative[e]	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5 - 250 mg/kg bw	Negative	Oh et al. (1999)
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative	Kerr et al. (1983)

NS = Not specified. [a] Without metabolic activation. [b] As calculated by Williams (2007). [c] With and without metabolic activation (source not specified in original monograph). [d] Inadequate detail available. [e] Sacrificed at 3 hours and 24 hours.

7. Clinical Studies & Other Reports in Humans

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated. The effects noted included glucose uptake, insulin secretion, and blood pressure (Geuns et al., 2003a). In South America, stevioside is used as a treatment for type 2 diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies of stevioside and further studies were recommended (WHO, 2006). Subsequently, several studies were conducted, and in 2009, JECFA reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included below.

a. Studies Summarized in 2006

In a study by Curi et al. (1986), aqueous extracts of 5 grams of *S. rebaudiana* leaves were administered to 16 volunteers at 6 hour intervals for three days, and glucose tolerance tests were

performed before and after the administration. Another six volunteers were given an aqueous solution of arabinose in order to eliminate possible effects of stress. The extract increased glucose tolerance and significantly decreased plasma glucose concentrations during the test and after overnight fasting in all volunteers.

In a multi-center randomized, double-blind, placebo-controlled trial of hypertensive Chinese men and women (aged 28–75 years), 60 patients were given capsules containing 250 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 750 mg of stevioside per day [equivalent to 11 mg per kg bw per day as calculated by FSANZ (2008)] and followed up at monthly intervals for one year. Forty-six patients were given a placebo. After 3 months, systolic and diastolic blood pressure in men and women receiving stevioside decreased significantly, and the effect persisted over the year. Blood biochemistry parameters, including lipids and glucose, showed no significant changes. Three patients receiving stevioside and one receiving the placebo withdrew from the study as a result of side effects (nausea, abdominal fullness, dizziness). In addition, four patients receiving stevioside experienced abdominal fullness, muscle tenderness, nausea, and asthenia within the first week of treatment. These effects subsequently resolved, and the patients remained in the study (Chan et al., 2000).

In a follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years), 85 patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1,500 mg of stevioside per day [equivalent to 21 mg per kg bw per day, as calculated by FSANZ (2008)]. Eighty-nine patients were given a placebo. During the course of study, three patients in each group withdrew. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean systolic and diastolic blood pressures were significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side effects (nausea, dizziness and asthenia), which led two patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

In a randomized, double-blind trial designed, 48 hyperlipidemic volunteers were recruited to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract used in this study was a product containing stevioside (73 ± 2%), rebaudioside A (24 ± 2%), and other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg per day, equivalent to 3.3 mg per kg bw per day assuming an average body weight of 60 kg), for 3 months. One subject from placebo group and three from treatment group failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum

concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes, and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regimen, in view of the similarity of effect between treatment and placebo (Anonymous, 2004a). In a follow-up study, 12 patients were given steviol glycosides extract in incremental doses of 3.25, 7.5, and 15 mg per kg bw per day for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004b).

In a paired cross-over study, 12 patients with type 2 diabetes were given either 1 gram of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 gram of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before, and for 240 minutes after, ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or observed by the investigators. Systolic and diastolic blood pressure was not altered by stevioside administration (Gregersen et al., 2004).

b. Studies Summarized in 2009

In a short-term study of stevioside in healthy subjects, 4 male and 5 female healthy volunteers (aged 21–29 years) were provided with capsules containing 250 mg stevioside (97% purity) to be consumed 3 times per day for 3 days (Temme et al., 2004). Doses, expressed as steviol, were 288 mg per day, or 4.4 mg per kg bw per day for females and 3.9 mg per kg bw per day for males. Twenty-four hour urine samples were taken before dosing on day 1 and after dosing on day 3. Fasting blood samples were taken before dosing on day 1, and six samples were taken at different time points on day 3 after dosing. Fasting blood pressure measurements were taken before the first capsule and at six different time intervals after the first dose. Urine was analyzed for creatinine, sodium, potassium, calcium, and urea. Blood was analyzed for plasma glucose, plasma insulin, alkaline phosphatase, alanine transaminase (ALT), glutamic-pyruvate transaminase (GPT), creatine kinase, and lactate dehydrogenase. The clinical analyses of blood, blood pressure, and urine showed no differences between samples taken before or after dosing.

In an unpublished double-blind, placebo-controlled trial study reviewed at the 68th JECFA meeting, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and 18.9% rebaudioside A, was administered to groups of type 1 (n = 8) and type 2 diabetics (n = 15), and non-diabetics (n = 15), 3 times daily for 3 months. Control groups with the same number of subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated hemoglobin (HbA1c), blood lipids, or renal or hepatic function. No adverse effects were reported. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Barriocanal et al., 2006, Barriocanal et al., 2008). The Committee previously noted that this product did not meet the proposed specification of "not less than 95% steviol glycosides" and that the study was conducted in a small number of subjects.

In a follow-up study, Barriocanal et al. (2008) evaluated the effects of steviol glycosides on blood glucose and blood pressure (BP) for three months in subjects with type 1 diabetes, subjects with type 2 diabetes, and subjects without diabetes and with normal/low-normal BP levels. Patients in each group received either 250 mg total dissolved solids (tds) steviol glycoside, stevioside, or placebo treatment. The purity of the steviol glycosides was ≥ 92%. Three months of follow up revealed no changes in systolic BP, diastolic BP, glucose, or glycated hemoglobin from baseline. In placebo type 1 diabetics, there was a significant difference in systolic BP and glucose. There were no adverse effects observed in either treatment group, and the authors concluded that oral steviol glycosides are well-tolerated and have no pharmacological effect.

A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana*. Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24 weeks or crude stevioside at consecutive doses of 3.75 mg per kg bw per day (7 weeks), 7.5 mg per kg bw per day (11 weeks) and 15 mg per kg bw per day (6 weeks). Comparison of patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Ferri et al., 2006). The product in this study also did not meet the proposed specification.

A placebo-controlled double-blind trial was carried out in 49 hyperlipidemic patients (aged 20–70 years, number of males and females not supplied) not undergoing treatment. The study was approved by the local ethics committee and complied with the principles of the Declaration of Helsinki. Individuals were divided into two groups, with 24 subjects receiving placebo capsules and 25 receiving capsules containing a dose of 50 mg steviol glycosides (70% stevioside, 20% Rebaudioside A), equivalent to 1.04 mg steviol per kg bw per day, using the mean body weight of the treatment group, 72.7 kg. Two capsules were taken before lunch, and two before dinner, each day for 90 days. Six subjects withdrew from the study, four in the placebo group and two in the test group. Self-reported adverse reactions were recorded, and fasting blood samples were taken at the end of the study and analyzed for alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides. No effects of treatment on ALT, AST, or GGT were found. Decreases in the total cholesterol and LDL were observed in both the stevioside group and the placebo group, which were not treatment related. No adverse effects were observed (Silva et al., 2006). The Committee noted at its 68th meeting that the product used in this study did not meet the proposed specification.

In a long-term, randomized, double blinded, placebo-controlled study, Jeppesen et al. (2006) investigated the efficacy and tolerability of oral stevioside in patients with type 2 diabetes. In this study, 55 subjects received 500 mg stevioside (purity unspecified), or placebo (maize starch), 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response, HbA1c, and fasting

blood glucose levels. HbA1c is an indicator of mean glucose levels and is used in identifying effects on the control of diabetes. No differences in lipids or blood pressure were observed. It is not clear whether this study was approved by the local ethics committee or met the requirements of the Declaration of Helsinki (Jeppesen et al., 2006).

Appendix 8 Summary of Studies on Steviol Glycosides Preparations That Are Primarily Rebaudioside A

Safety Data on Rebaudioside A[11]

Since 2008, several well-designed toxicology studies that followed the current regulatory and scientific guidelines for such studies have been reported on purified rebaudioside A, although it is uncertain whether or not these studies were considered by JECFA during its 2008 deliberations. These recent investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies. These studies confirm that rebaudioside A is metabolized similarly to other steviol glycosides, and they exhibited an absence of toxicological effects in the key studies reviewed by JECFA. It should be noted that rebaudioside A, as the steviol glycoside with high sweetness intensity and relatively high prevalence in the stevia leaves, remains an active topic of scientific research. For example, a study found in a recent literature search examined the anti-hyperglycemic activity of rebaudioside A in diabetic rats (Saravanan and Ramachandran, 2012). These investigators found that the effects of streptozotocin-induced diabetes on glucose and insulin levels were at least partially reversed in a dose-dependent manner with oral administration of rebaudioside A at doses in the range of 50-200 mg per kg bw. The doses used are 10-40 times higher than expected from the use of rebaudioside A as a sweetener. The known anti-hyperglycemic activity of steviol glycosides led JECFA to require clinical studies at reasonably high doses to show that—at levels used in food—there would be no effect on glucose homeostasis or blood pressure in human consumers. The clinical studies described below on rebaudioside A (Maki et al., 2008a, Maki et al., 2008b) the lack of these pharmacological effects of rebaudioside A at expected levels of consumption.

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Studies investigating the ADME of extracts from stevia are available on stevioside, Reb A, and other steviol glycosides. Data evaluating the absorption and fate of these extracts from various animal species and humans indicate that one can extrapolate these results from rats to humans. Stevioside is metabolized to steviol *via* intestinal microflora, and the absorption of stevioside after oral administration has been shown to be very low (Koyama et al., 2003, Geuns et al., 2003b, Geuns et al., 2003a).

[11] Questions about the safety of rebaudioside A were previously raised by Huxtable, R. J. (2002) 'Pharmacology and toxicology of stevioside, rebaudioside A, and steviol. ', in Kinghorn, A.D., (Ed.) (ed.) *Stevia: The Genus of Stevia.* NY: Taylor and Francis, Inc., and Kobylewski, S. and Eckhert, C. D. (2008) *Toxicology of Rebaudioside A: A Review.* University of California at Los Angeles. Available at: Originally accessed at www.cspinet.org/new/200808281.html.. Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels, have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304.

Studies investigating the hydrolysis of steviol glycosides by intestinal microflora have demonstrated that both stevioside and Reb A are hydrolyzed to steviol following *in vitro* incubation with various cecal microflora (Wingard Jr et al., 1980, Hutapea et al., 1997, Gardana et al., 2003, Geuns et al., 2003a). In addition, the *in vitro* hydrolysis of Reb A to steviol was found to be slower than that of stevioside (Koyama et al., 2003), which is thought to be partly due to the presence of one additional glucose moiety and to differences in structural complexities. Koyama et al. (2003) suggest that the major pathway for Reb A is conversion to stevioside with a minor pathway of conversion to Reb B prior to being ultimately converted to steviol. Stevioside is further converted to steviolbioside, steviolmonosides, and finally steviol, with glucose being released with each subsequent hydrolysis.

In three recently completed studies, absorption and fate of rebaudioside A were systematically investigated in rats and humans.

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabeled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A.

Roberts and Renwick (2008) identified free steviol (82 to 86%), steviol, glucuronide (10 to 12%), and two unidentified metabolites (5-6%) in rat plasma following treatment with either stevioside or Reb A eight hours post-oral administration. A comparable pharmacokinetic profile was noted following oral treatment of rats with radiolabeled Reb A or stevioside, with the time of maximum plasma concentration (T_{max}) for radioactivity ranging between 2 and 8 hours. In comparison, steviol T_{max} for plasma was noted within 30 minutes of oral administration. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two unidentified metabolites. It is believed that this delay between the occurrence of radioactivity in the plasma and time of administration of steviol glycosides is due to the fact that the Reb A and stevioside are first cleaved to steviol before absorption.

Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. Following elimination in the bile, steviol is available to be released again from its conjugated form by microflora activity and may enter enterohepatic circulation. Consequently, free and conjugated steviol are secreted in the feces along with any unhydrolyzed fraction of the administered glycosides. Following Reb A treatment, significant amounts of unchanged rebaudioside A (29% in males and 19% in females) and stevioside (3% in males and 4% in females) were excreted in the feces. Following oral stevioside administration, unchanged stevioside was excreted in rat feces. Other unidentified metabolites are also present in fecal samples of rats treated with either glycoside. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with ~60% of the radioactivity eliminated in the feces within 48

hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide (Roberts and Renwick, 2008).

In summary, Roberts and Renwick (2008) found that steviol was the predominant component found in plasma samples after oral administration of Reb A, stevioside, and steviol in rats. Lower amounts of steviol glucuronide(s) and one or two unidentified metabolites were also found. The majority of all samples were found to be excreted rapidly---primarily in the feces---within 48 hours. This is in agreement with the previous *in vitro* hydrolysis data that indicated that both Reb A and stevioside are metabolized to steviol by intestinal microflora. The predominant compound detected in the bile was steviol glucuronide, while the prominent material in the intestine was steviol, which the authors suggest indicates that deconjugation occurs in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median T_{max} values of 12.0 and 8.00 hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for each compound. Administration of rebaudioside A resulted in a significantly (~22%) lower steviol glucuronide geometric mean C_{max} value (1,472 ng per mL) than administration of stevioside (1,886 ng per mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30,788 ng*hr per mL) was approximately 10% lower than after administration of stevioside (34,090 ng*hr per mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72-hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide, indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). The investigators concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans, with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety, or vital signs (Wheeler et al., 2008).

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2,000 mg per kg bw per day (Sloter, 2008a). Extremely low levels of rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2,000 mg per kg bw per day of rebaudioside

A, with mean plasma concentrations of approximately 0.6 and 12 μg per mL, respectively. Estimates of absorbed dose for rebaudioside A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal rebaudioside A and measured hydrolysis products, expressed as Total Rebaudioside A Equivalents, compared to daily administered dose results in an estimated dose recovery of approximately 84%.

2. Subchronic Toxicity Studies

Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9,938 and 11,728 mg per kg bw per day for males and females, respectively) for 4 weeks, or 50,000 ppm (4,161 and 4,645 mg per kg bw per day for males and females, respectively) for 13 weeks, did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000, and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000, and 50,000 ppm. In high-dose male and females groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the feed were observed. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. No significant changes in other clinical pathology results, organ weights, and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm, or approximately 4,161 and 4,645 mg per kg bw per day in male and female rats, respectively (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1,000, and 2,000 mg per kg bw per day were tested in Crl:CD(SD) rats (Nikiforov and Eapen, 2008, Eapen, 2007). Each group consisted of 20 animals per sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. There were no treatment-related macroscopic, organ weight or microscopic findings. Significantly lower body weight gains were noted in the 2,000 mg per kg bw per day group in males but not females. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control group value, the investigators did not consider this result to be adverse. The decrease was most likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥ 2,000 mg per kg bw per day.

A 6-month dietary toxicity study in Beagle dogs (4 per sex per group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1,000, or

2,000 mg per kg bw per day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators concluded that no systemic toxicity of rebaudioside A was observed at dosage levels up to 2,000 mg per kg bw per day and the assigned NOAEL was ≥ 2,000 mg per kg bw per day.

In addition, a 90-day subchronic toxicity study was conducted in Sprague-Dawley rats using fermentation-derived Rebaudioside A, where no systemic or local toxicity was observed in rats dosed at 500 to 2,000 mg per kg bw per day. All test animals survived to scheduled necropsy (Rumelhard et al., 2016).

3. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage, and deoxyribonucleic acid (DNA) strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al., 1985, Nakajima, 2000a, Nakajima, 2000b, Sekihashi et al., 2002). These studies were critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside A, including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006), and a mouse micronucleus study (Krsmanovic and Huston, 2006), submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Furthermore, Williams and Burdock (2009) also reported lack of genotoxicity in another set of published studies that included *in vitro* mutagenicity assays with *Salmonella, E. coli,* and mouse lymphoma cells. These investigators also reported lack of *in vitro* clastogenic effects in Chinese hamster V79 cells, and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis.

The recent evaluation of fermentation-derived rebaudioside A demonstrated a similar safety profile to plant-derived rebaudioside A. Rumelhard et al. (2016) reported that fermentation-derived rebaudioside A was not mutagenic in the bacterial reverse mutation assay, nor was it found to be clastogenic or aneugenic in the *in vitro* micronuleus assay. The similarity of the safety profile observed between plant-derived and fermentation-derived rebaudioside A further supports the applicability of the safety assessments to other steviol glycoside preparations.

The key mutagenicity testing results for rebaudioside A are summarized in Table 8-1.

Table 8-1. Mutagenicity & Genotoxicity Studies on Rebaudioside A

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
Bacterial Mutagenicity	5 *Salmonella* strains with & without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1,500 & 5,000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 *Salmonella* strains & 1 *E. coli* strain with & without exogenous metabolic activation system	Reb A	95.6	Up to 5,000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Bacterial Mutagenicity	4 *Salmonella* strains & 1 *E. coli* strain with and without exogenous metabolic activation system	Fermentation-derived Reb A	≥ 95%	Up to 5,000 µg per plate	No mutagenic response	Rumelhard et al. (2016)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence & presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1,000, 2,000, 3,000, 4,000 & 5,000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence & presence of exogenous metabolic activation system	Reb A	95.6	Up to 5,000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Human Lymphocytes	Human lymphocytes in absence & presence of exogenous activation system	Fermentation-derived Reb A	≥ 95%	Up to 5,000 µg/mL	Not clastogenic or aneugenic	Rumelhard et al. (2016)
Chromosome Aberration	Human lymphocytes in absence & presence of exogenous metabolic activation system	Reb A	95.6	Up to 5,000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male & 5 female ICR mice	Reb A	99.5	500, 1,000 & 2,000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male & 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2,000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52%;	250 – 2,000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
			Reb A, 22%			
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2,000 mg/kg bw/ day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified.

[a] Sacrificed at 3 hours and 24 hours.

[b] With or without metabolic activation (source not specified in original monograph).

[c] Sacrificed at 30 hours after 2nd administration.

4. Reproductive & Developmental Toxicity Studies

In a two-generation reproductive toxicity study, rebaudioside A (97% purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry et al., 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F_0 or F_1 generations. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm, and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm, or 2,048 to 2273 mg per kg bw per day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter, 2008a, Sloter, 2008b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30 per sex per group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter, 2008a). For the F_0 and F_1 generations, rebaudioside A doses were 0, 500, 1,000, and 2,000 mg per kg per day. At initiation of study, F_0 animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F_1 generation following weaning [beginning on postnatal day (PND) 21]. The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental

systemic and reproductive toxicity, a dose level ≥ 2,000 mg per kg bw per day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered *via* gavage were investigated. Rebaudioside A administration did not affect intrauterine growth and survival, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity, a dose level ≥ 2,000 mg per kg bw per day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

5. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A, at a dose of 1,000 mg per day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP), were investigated (Maki et al., 2008a). Subjects were predominantly female (76% rebaudioside A and 82% placebo) with a mean age of ~41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the rebaudioside A and placebo groups, respectively. Compared with placebo, administration of rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The investigators concluded that consumption of 1,000 mg per day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In another trial, effects of 16 weeks of consumption of 1,000 mg per person per day rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki et al., 2008b). Changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A (0.11 ± 0.06%, mean ± standard error) and placebo (0.09 ± 0.05%; p = 0.355) groups. Similarly, no significant ($p > 0.05$ for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg per dL and 11.2 ± 4.5 mg per dL), insulin (1.0 ± 0.64 µU per mL and 3.3 ± 1.5 µU per mL), and C-peptide (0.13 ± 0.09 ng per mL and 0.42 ± 0.14 ng per mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1,000 mg per person per day rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

6. Safety of Rebaudioside A

There have been a significant number of studies regarding the safety and toxicity of rebaudioside A, including many that have been published since the two initial GRAS notifications were submitted to FDA by Cargill (GRN 253) and Merisant (GRN 252). These, and some other unpublished studies, formed the basis of the two initial GRAS notifications to FDA by Cargill (GRN 253) and

Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on rebaudioside A were conducted. Even before these new studies were completed, and as noted in the previous section, JECFA concluded that 7 (which was later expanded to 9) common steviol glycosides are deemed to be safe for use as sweetener preparations when present in any combination, as long as a combined purity of 95% or more was established.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself, and to demonstrate that rebaudioside A is handled pharmacokinetically similarly to stevioside in rats and humans. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group conducted three mutagenicity assays on rebaudioside A that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure.

In a review article, Carakostas et al. (2008) summarized the most recent Cargill research program findings on rebaudioside A, as follows:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- While studies with Reb A indicated slight gastrointestinal (GI) absorption of the glycoside *per se*, the predominant metabolic pathway is comparable to that of stevioside and the use of the ADI established by JECFA, which was determined on studies employing stevioside as the main component, can be used as the ADI for rebaudioside A.
- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

The consumption estimates described by JECFA, Renwick (2008), and the GRN 252 and GRN 253 Expert Panels very conservatively represent a potential high user of Rebaudioside A if this non-nutritive sweetener becomes widely available in food.

Regarding the available aggregate safety information, multiple qualified entities have concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity, as defined by JECFA specifications, has been properly determined to be 4 mg per kg bw per person as steviol equivalents, which corresponds to 12 mg per kg bw per day for rebaudioside A, on a dry weight basis. Unwanted pharmacological effects are not likely to occur at this level and, moreover, high consumers of rebaudioside A are not likely to exceed this level. Therefore, the JECFA-derived ADI was adopted as a safe exposure for rebaudioside A and the corresponding food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS).

JECFA---which is composed of dozens of scientists that are internationally known experts on food ingredient safety---has established ADIs for food ingredients over the last 40 years. Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.

The studies conducted by Cargill provided significant insight into the pharmacokinetics of rebaudioside A, while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rat. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

Appendix 9 Studies on Principal Metabolite: Steviol

Studies on Principal Metabolite: Steviol

In a number of studies, steviol, the principal mammalian metabolite of stevioside, has been investigated for its safety. The results of these studies are summarized below.

1. Acute Toxicity Studies

The oral LD_{50} of steviol (purity, 90%) in male and female mice and rats was reported to be > 15 grams per kg bw. In this study, only one of 15 animals died within 14 days of administration. The LD_{50} values in hamsters given steviol orally were 5.2 grams per kg bw in males and 6.1 grams per kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkac et al., 1997).

2. Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1,000 mg per kg bw per day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg per kg bw per day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

3. Mutagenicity & Genotoxicity Studies

In a number of studies mutagenicity and genotoxicity of steviol has been investigated. These studies reviewed by JECFA are summarized in Table 9-1.

Table 9-1. Mutagenicity & Genotoxicity Studies on Steviol

	In Vivo/In Vitro	System	Test Sample Purity	Author Conclusion	Results and Remarks
Sekihashi et al. (2002)[a]	*In Vivo/In Vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1,000 or 2,000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical *in vivo* experiment with stevia extract performed, which also gave negative results.
Oh et al. (1999)[b]	*In Vivo?*	Cell Mutation and DNA damage	Not reported	Negative	Steviol gave negative results for cell mutation and DNA damage in cultured cells.
Matsui et al. (1996)[c]	*In Vivo?*	Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts)	Not reported	Positive	Gene mutation and chromosomal aberration found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.
Terai et al. (2002)[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Steviol found to be mutagenic in Aroclor-induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture.
Temcharoen et al. (1998)[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in *S. typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanichpak et al. (1997)[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *S. typhimurium, E. coli WP2, uvrA/PKM101* and rec assay using *B. subtilis* even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported.
Matsui et al. (1996)[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of *E. coli*. The chromosomal DNA of TM677 and steviol-induced TM677 mutants

	In Vivo/In Vitro	System	Test Sample Purity	Author Conclusion	Results and Remarks
					digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al. (1996)[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Both	Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation.
Procinska et al. (1991)[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.
Compadre et al. (1988)[a]	*In Vitro*	Bacterial Mutagenicity, Mass Spec	Not Reported	Positive	Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be direct-acting mutagen. Magnitude of increase over control in assay not discussed.
Pezzuto et al. (1985)[d]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *S. typhimurium* TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicity dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors concluded that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17.
Temcharoen et al. (2000)[c]	*In Vivo*	Micronucleus (rat)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Temcharoen et al. (2000)[c]	*In Vivo*	Micronucleus (mouse)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Matsui et al. (1996)[a]	*In Vivo*	Micronucleus (mouse)	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Temcharoen et al. (2000)[c]	*In Vivo*	Micronucleus (hamster)	90%	Negative	Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.

[a] Abstract only. [b] As reported in WHO (2006). [c] As reviewed by Geuns (2003). [d] Full article.

4. Endocrine Disruption Studies

Shannon et al. (2016) investigated the endocrine disrupting potential of stevioside, rebaudioside A, and steviol in a series of *in vitro* bioassays. Steviol was reported to 1) antagonize progesterone nuclear receptor transcriptional activity; 2) increase progesterone production; and 3) induce an agonistic response on the progesterone receptor of sperm cells (Catsper). While the authors concluded that *Stevia* might not qualify as a safer alternative to sugar or synthetic sweeteners, it is important to note that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at the receptor level. Furthermore, no adverse effects were observed in the reproductive studies.

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