



SAMI LABS LIMITED



Report on Ocular and Dermal Irritation of Boswellin CG Utilizing the Irritection® Assay System

Study Report # : IR 115
Test Article : Boswellin CG, Lot # C00648I
Manufactured by : Sami Labs Ltd.
Sponsor : Sabinsa Corporation
Study Completion Date : March 22nd 2005
Testing Laboratory : Department of Biochemical Evaluation.
Sami Labs Ltd.

EXECUTIVE SUMMARY

Boswellin CG was evaluated with the Irritection Assay System in order to predict its potential to cause ocular and dermal irritation. The ocular results demonstrated that the sample was a Minimal irritant. The dermal results indicated that it was a Non-irritant.

Study Scientists

Sadia Gul Afroze and Sushmitha Anand

Date

25/5/05

Signature

Sadia Gul Afroze

Sushmitha Anand
25.05.05

Scientist Coordinated the Study

Geetha. K.G

Date

26/5/05

Signature

Geetha K.G

Executive Vice President R&D

Dr. S. Natarajan

Date

29.5.05

Signature

S. Natarajan

STUDY OBJECTIVE

Boswellin CG was evaluated with the Irritection[®] Assay System in order to predict its potential to cause ocular and dermal irritation.

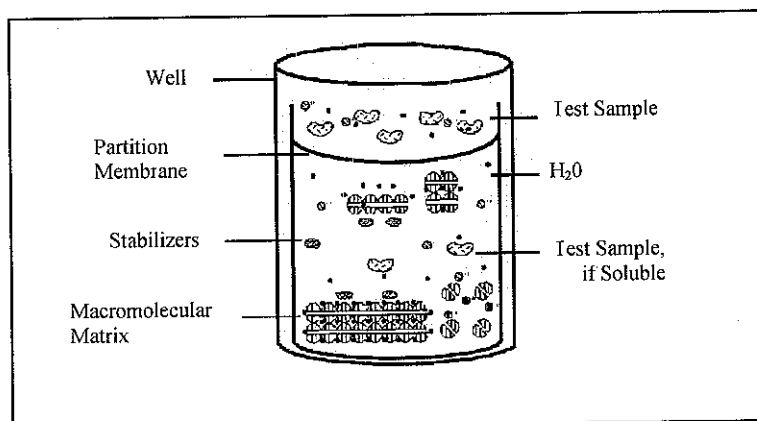
To achieve this objective, standard Concentration dependent dose-response studies were performed with the Ocular and Dermal Irritection test methods

BACKGROUND

The proprietary Ocular and Dermal Irritection assays are standardized and quantitative *in vitro* acute ocular and dermal irritation tests which utilize changes of relevant macromolecules to detect, rank and predict acute ocular and dermal irritancy of chemicals and chemical formulations.

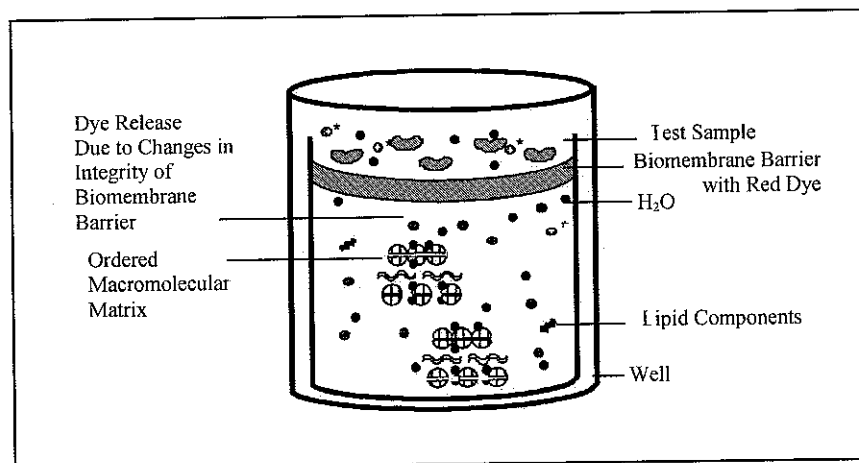
The Ocular Irritection assay, depicted schematically in Figure 1 below, provides significant advances over the *in vivo* Draize test method. The Draize eye irritation assay has been criticized because of the large variability of results obtained from different laboratories that have analyzed the same specimen

Figure 1. The Ocular Irritection Model



The Dermal Irritection assay, depicted schematically in Figure 2, is based on the principle that chemical compounds will promote measurable changes in target biomolecules and macromolecular structures. Previous studies have clearly demonstrated that the processes of protein denaturation and disaggregation that are induced in this *in vitro* assay mimic the effects that are produced when these types of irritants are applied to the skin. Consequently, this *in vitro* test may be employed to predict the *in vivo* toxic effects of chemicals and formulations.

Figure 2. The Dermal Irritection Model



The quantitative Ocular and Dermal Irritection *in vitro* assays have been found to be highly reproducible. Of even greater relevance, the Irritection assay methods can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, these tests serve as extremely useful screening tools that facilitate all stages of raw material selection, formulation development and final product selection.

MATERIALS/METHODS

The Ocular and Dermal Irritection assays are quantitative *in vitro* test methods that mimic acute ocular and dermal irritation tests. To perform the Ocular Irritection standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. To perform the Dermal Irritection standardized assay, the test sample is applied to a similar synthetic biobarrier that is coated with a dye-containing keratin-collagen matrix. Following application, the sample is absorbed by and permeates through this synthetic bio barrier to gradually come into contact with a proprietary solution containing highly ordered globulins and glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. With the Ocular Irritection test, turbidity may be detected spectrophotometrically at a wavelength of 405 nm. With the Dermal Irritection test, turbidity as well as the dye that has been dissociated from the biobarrier during transit of the applied sample may be detected spectrophotometrically at a wavelength of 450 nm.

The ocular irritancy potential of a test sample is expressed as an Irritection Draize Equivalent (IDE), whereas the dermal irritancy potential of a test sample is expressed as a Human Irritancy Equivalent (HIE). These scores are defined by comparing the increase in optical density (OD_{405/450}) produced by the test material to a standard curve that is constructed by measuring the increase in OD produced by a set of Calibration substances. These Calibrators have been selected for use in these tests because their irritancy potential has been previously documented in a series of *in vivo* investigations. The predicted *in vivo* classification, based on these scoring systems, is shown in Tables 1 and 2.

Table 1. Relationship of Irritection Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritection Test Method.

Irritection Draize Equivalent (IDE) Score	Predicted Ocular Irritancy Classification
0.0 - 12.5	Minimal Irritant
12.5 - 30.0	Mild Irritant
30.0 - 51.0	Moderate Irritant
>51.0	Severe Irritant

Table 2. Relationship of Human Irritancy Equivalent (HIE) Score to Irritancy Classification for the Dermal Irritection Test Method.

Human Irritancy Equivalent (HIE)	Predicted Dermal Irritancy Classification
0.00 - 0.90	Non-Irritant
0.90 - 1.20	Non-Irritant/Irritant
1.20 - 5.00	Irritant

A detailed description of the Ocular and Dermal Irritection test procedures may be found in InVitro International's Irritection[®] Assay System Instruction Manual. All data are calculated and analyzed via a computer program which determines assay result acceptance based upon qualification parameters defined in the program. In general, the program has been designed to accept sample data as qualified if the following criteria are met: the OD values of Calibrators and internal Quality Control samples fall within previously specified ranges; sample blanks are less than 500 OD units; the net sample OD is greater than -15; and an Inhibition Check is negative.

RESULTS

The results of this analysis provided a predicted *in vivo* classification for the test sample. The software printouts are included in Assay Report.

Tables 1 and 2 present a summary of results for the sample studied.

Table 1. Summary of Ocular Irritation Results

Lot #	Sample	Concentration (mg)	IDE Score	Predicted Ocular Irritancy Classification
C00648I	Description: Off White powder	50	9.7*	Minimal Irritant
		75	6.2	Minimal Irritant
		100	6.4	Minimal Irritant
		125	7.5	Minimal Irritant

*Maximum Qualified Score

Table 2. Summary of the Dermal Irritation Results

Lot#	Sample	Concentration (mg)	HIE Score	Predicted Dermal Irritancy Classification
C00648I	Description: Off White powder	50	0.38	Non-Irritant
		75	0.42*	Non-Irritant
		100	0.29	Non-Irritant
		125	0.24	Non-Irritant

* Maximum Qualified Score

DISCUSSION

Boswellin CG was evaluated with the Irritation Assay System in order to predict its potential to cause ocular and dermal irritation.

Standard Volume-dependent dose-response studies were performed with the Ocular and Dermal Irritation test methods. The following doses of sample were applied for analysis: 50, 75, 100, and 125mg. The ocular results demonstrated that the Boswellin CG was classified as a Minimal irritant with an IDE score of 9.7. The dermal results indicated that it was predicted to be a non-irritant with an HIE score of 0.42.

In summary, the Irritation test method successfully classified Boswellin CG as Minimal irritant for the ocular irritation potential and as Non-irritant for the dermal irritation potential.

ASSAY REPORT - ORIGINAL

Sample Description	: Boswellin CG	Date	: 03/22/05
Sample Number	: 39	Time	: 15:57:09
Product Type	:	Technician Name	: Sadia Gul Afroze
Assay Method	: Ocular	Kit Lot Number	: IO-092503
Protocol	: Cosmetic- IO 092503	Reagent temperature	: 27.0
Incubation Time	: 24.0 hours	Reagent pH Before Activation	: 8.11
Plate Layout	: 5 Samples/4 Volumes	Reagent pH After Activation	: 6.59
Instrument Type	: Dynex MRX	Sample pH	:
Wavelength	: 405nm	Assay Number	: 13
Comment	: C00648T	Assay Qualification	: Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
50 mg	224 [126]	-17	241	9.7	Minimal	Examine dose curve
75 mg	138 [40]	-17	155	6.2	Minimal	Qualified
100 mg	144 [46]	-16	160	6.4	Minimal	Qualified
125 mg	174 [76]	-12	186	7.5	Minimal	Qualified

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	115** [213]	0.0	42 - 179	Range qualified: Sub
Cal 1	311** [213]	12.5	116 - 410	Range qualified
Cal 2	850** [752]	30.0	550 - 1483	Range qualified
Cal 3	1919** [1821]	51.0	1629 - 2715	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	390 [292]	15.1	6.6 - 23.0	Range qualified
QC 2	719 [621]	25.7	25.7 - 38.9	Range qualified

Sample Inhibition Check Results:

Dose / Inhibition Check OD
125 mg / 1930

* Mean value from assay data history

** Mean value from protocol defaults or adjusted value due to calibrator zero substitution

[] Value before substitution

PROTOCOL REPORT - ORIGINAL

Assay Method : Ocular
Protocol Name : Cosmetic- IO 092503

DOS File Name : P6114156 RPR
Date / Time : 11/16/04 11:44:59

Score Precision : 1
Number of Quality Controls Used in Assay : 2
Maximum Number of Out-of-Range Calibrators and Quality Controls : 1
History Recording Enabled : Yes
Substitution From History Enabled : No
10 % Borderline Classifications : Yes, based on calibrator class scores

Cal Designation	Name	Irritancy Score	Mean OD	Range Limit (OD)
Cal 0	Calibrator 0	0.0	115	42 - 179
Cal 1	Calibrator 1	12.5	262	116 - 410
Cal 2	Calibrator 2	30.0	1016	550 - 1483
Cal 3	Calibrator 3	51.0	2172	1629 - 2715

QC Designation	Name	Irritancy Score	Range Limit (Score)
QC 1	Quality Control1	14.8	6.6 - 23.0
QC 2	Quality Control2	32.3	25.7 - 38.9

Irritation Classification	Range Limit (Score)
Minimal	0.0 - 12.5
Mild	12.5 - 30.0
Moderate	30.0 - 51.0
Severe	51.0 - 80.0

Qualification Test	Enabled?	Qualification Requirements
Calibrators	Yes	Range
Quality Controls	Yes	Range
Blanks	Yes	Min -100 Max 500 BBF 20 BOF 40
Net OD	Yes	Min -15
Inhibition	Yes	
Dose Curve	Yes	Low 200 Mid 700 LF 20 LOF 30 MF 40 MOF 60
Concentration Curve	Yes	Low 200 Mid 700 LV 30 MV 60

Dose Curve Requirements	
Method Used to Determine the Highest Qualified Dose	Empirical
Minimum Number of Qualified Doses	3
Consecutive Qualified Doses Required	No
Doses Higher than Any Failed Dose Also Fail	No
Require Highest Dose to Qualify	No
Doses with Increasing Net ODs Qualify	Yes
Doses Above the Highest Qualified Dose Fail	Yes
Include Unqualified Doses on Curve	Yes

Concentration Curve Requirements	
Method Used to Determine the Highest Qualified Concentration	Empirical
Concentrations Above the Highest Qualified Concentration Fail	Yes
Include Unqualified Concentrations on Curve	Yes

Plate Layout Name : 5 Samples/4 Volumes
Number of Plates : 1

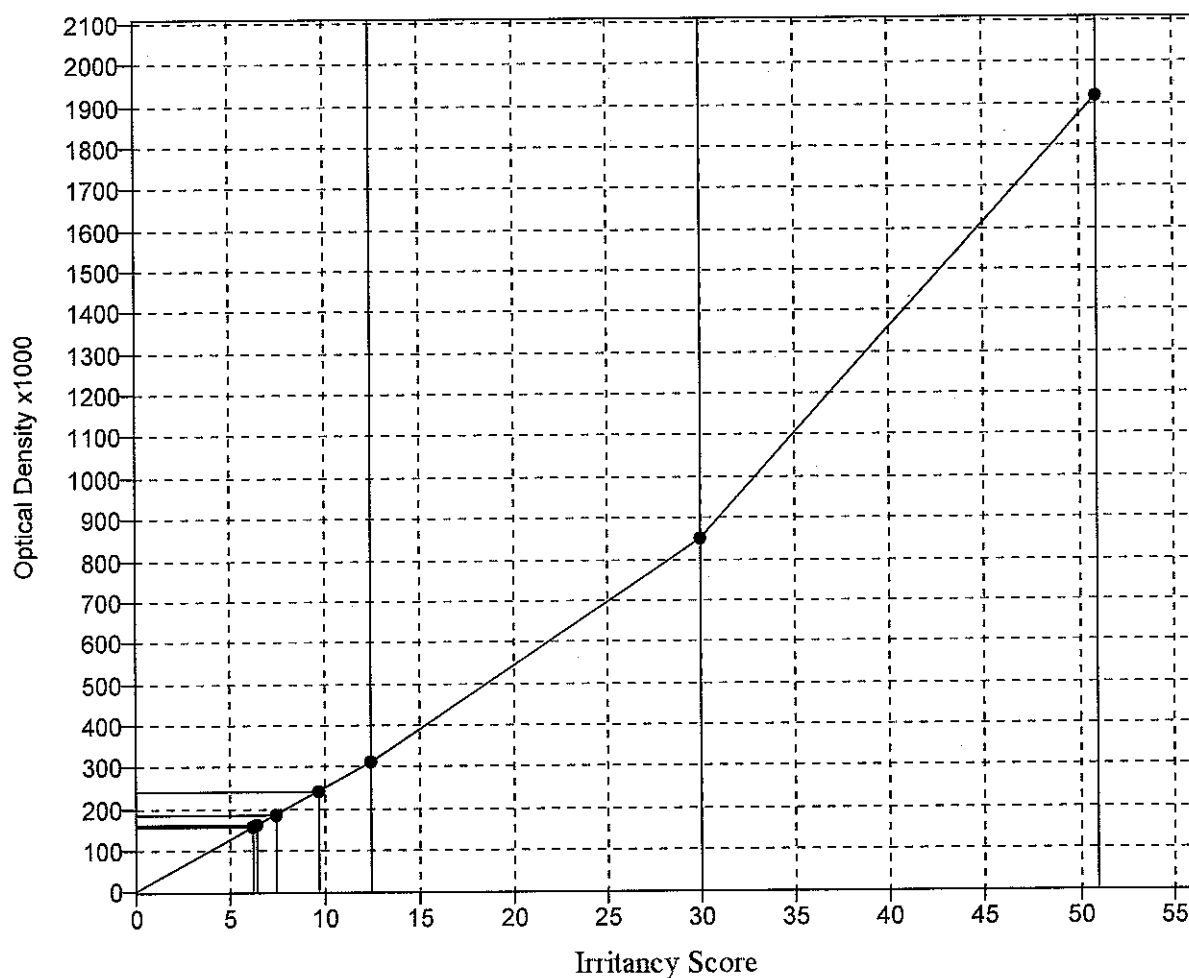
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CALIBRATION CURVE - ORIGINAL

Sample Description : Boswellin CG
 Sample Number : 39
 Assay Method : Ocular
 Protocol : Cosmetic- IO 092503
 Date : 03/22/05
 Technician : Sadia Gul Afroze

Designation	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification
Cal 0	115				
Cal 1	311				
Cal 2	850				
Cal 3	1919				
50 mg	224	-17	241	9.7	Minimal
75 mg	138	-17	155	6.2	Minimal
100 mg	144	-16	160	6.4	Minimal
125 mg	174	-12	186	7.5	Minimal

Calibration Curve



DOSE RESPONSE CURVE - ORIGINAL

Sample Description : Boswellin CG
 Sample Number : 39
 Assay Method : Ocular
 Protocol : Cosmetic- IO 092503
 Date : 03/22/05
 Technician : Sadia Gul Afroze

Designation	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification
Cal 0	115				
Cal 1	311				
Cal 2	850				
Cal 3	1919				
50 mg	224	-17	241	9.7	Minimal
75 mg	138	-17	155	6.2	Minimal
100 mg	144	-16	160	6.4	Minimal
125 mg	174	-12	186	7.5	Minimal

Dose Response Curve

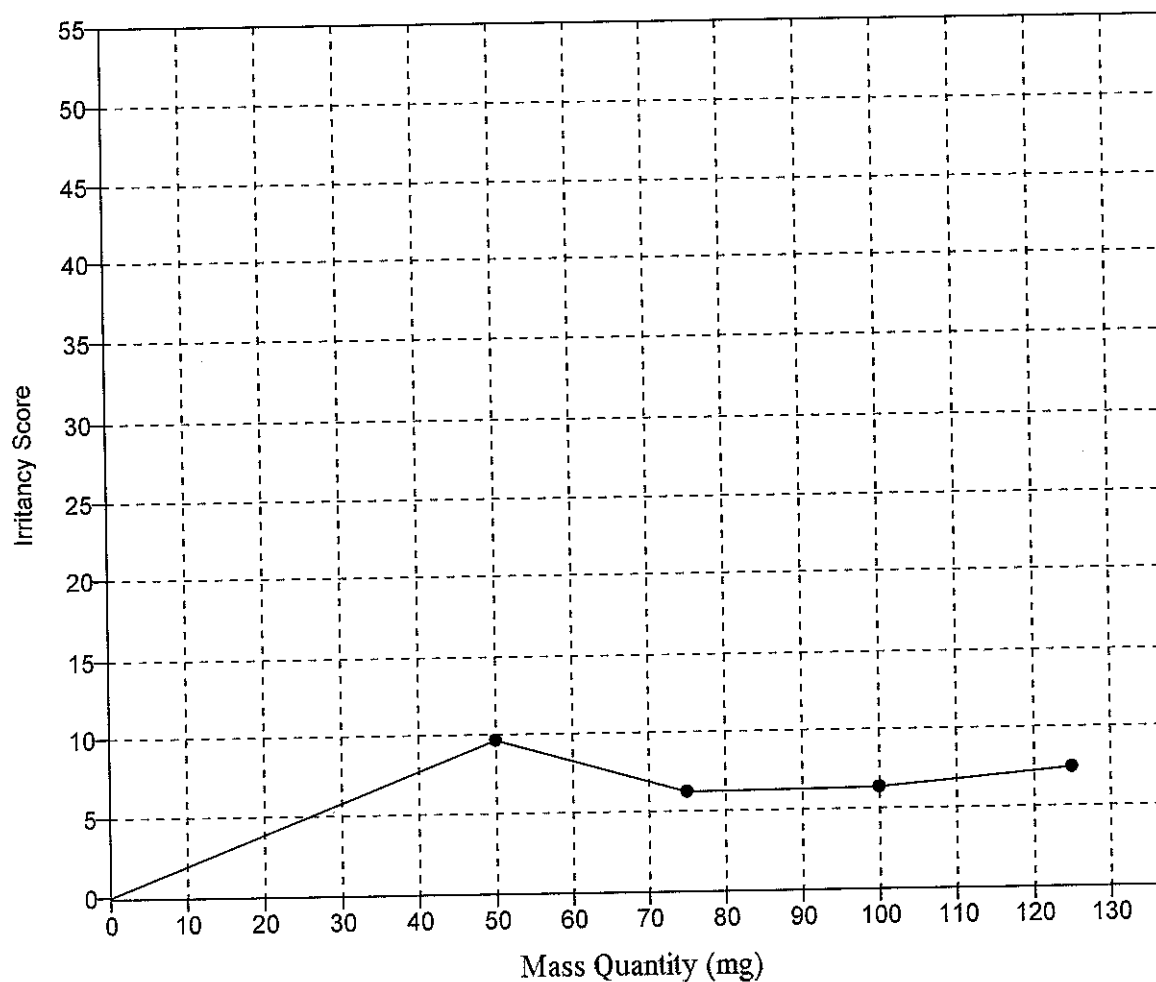


PLATE DATA - ORIGINAL

Sample Description: Boswellin CG
Sample Number : 39
Assay Method : Ocular
Protocol : Cosmetic- IO 092503
Date : 03/22/05
Technician : Sadia Gul Afroze

Blank Value : 65

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	9	344	4	328	12	318	-17	339	-2	354	-11
B	-18	-19	-19	-18	-20	-19	-19	-19	-17	-19	-19	-19
C	213	12	332	10	343	71	320	-17	253	11	357	2034
D	-19	-17	-17	-20	-20	-18	-17	-18	-15	-16	-14	-19
E	426	40	461	27	405	193	421	-16	259	24	363	505
F	-19	-20	-19	-19	-19	-18	-17	-16	-18	-16	-19	-19
G	965	95	615	77	514	594	974	-12	289	40	443	834
H	-20	-20	-20	-21	-22	-21	-20	-21	-19	-20	-20	-20

ASSAY REPORT - ORIGINAL

Sample Description	: Boswellin C G	Date	: 03/19/05
Sample Number	: 31	Time	: 11:41:10
Product Type	: Solid	Technician Name	: Sushmitha
Assay Method	: Dermal	Kit Lot Number	: ID 052604
Protocol	: Cosmetic - ID 052604	Reagent temperature	: 25.0
Incubation Time	: 24.0 hours	Reagent pH Before Activation	: 9.75
Plate Layout	: 5 Samples/4 Volumes	Reagent pH After Activation	: 7.95
Instrument Type	: Dynex MRX	Sample pH	:
Wavelength	: 450nm	Assay Number	: 22
Comment	: C00648T	Assay Qualification	: Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
50 mg	55	-4	59	0.38	Non-Irritant	Qualified
75 mg	57	-8	65	0.42	Non-Irritant	Qualified
100 mg	42	-4	46	0.29	Non-Irritant	Qualified
125 mg	35	-3	38	0.24	Non-Irritant	Qualified

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	59	0.00	0 - 100	Range qualified
Cal 1	156	1.00	60 - 260	Range qualified
Cal 2	813	2.00	330 - 886	Range qualified
Cal 3	850	4.00	810 - 1430	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	68	0.44	0.11 - 0.95	Range qualified
QC 2	85	0.54	1.95 - 3.60	Range test failed

Sample Inhibition Check Results:

Dose / Inhibition Check OD
125 mg / 1480

* Mean value from assay data history

** Mean value from protocol defaults or adjusted value due to calibrator zero substitution

[] Value before substitution

PROTOCOL REPORT - ORIGINAL

Assay Method : Dermal
Protocol Name : Cosmetic - ID 052604

DOS File Name : P6115157 RPR
Date / Time : 11/16/04 12:01:04

Score Precision : 2
Number of Quality Controls Used in Assay : 2
Maximum Number of Out-of-Range Calibrators and Quality Controls : 1
History Recording Enabled : Yes
Substitution From History Enabled : No
10 % Borderline Classifications : No, based on calibrator class scores

Cal Designation	Name	Irritancy Score	Mean OD	Range Limit (OD)
Cal 0	Calibrator 0	0.00	50	0 - 100
Cal 1	Calibrator 1	1.00	160	60 - 260
Cal 2	Calibrator 2	2.00	608	330 - 886
Cal 3	Calibrator 3	4.00	1120	810 - 1430

QC Designation	Name	Irritancy Score	Range Limit (Score)
QC 1	Quality Control1	0.53	0.11 - 0.95
QC 2	Quality Control2	1.65	1.95 - 3.60

Irritation Classification	Range Limit (Score)
Non-Irritant	0.00 - 0.90
Non-Irr /Irr.	0.90 - 1.20
Irritant	1.20 - 5.00

Qualification Test	Enabled?	Qualification Requirements
Calibrators	Yes	Range
Quality Controls	Yes	Range
Blanks	Yes	Min -50 Max 500 BBF 20 BOF 40
Net OD	Yes	Min -15
Inhibition	Yes	
Dose Curve	Yes	Low 200 Mid 700 LF 20 LOF 30 MF 40 MOF 60
Concentration Curve	Yes	Low 200 Mid 700 LV 30 MV 60

Dose Curve Requirements	
Method Used to Determine the Highest Qualified Dose	Empirical
Minimum Number of Qualified Doses	3
Consecutive Qualified Doses Required	No
Doses Higher than Any Failed Dose Also Fail	No
Require Highest Dose to Qualify	No
Doses with Increasing Net ODs Qualify	Yes
Doses Above the Highest Qualified Dose Fail	Yes
Include Unqualified Doses on Curve	Yes

Concentration Curve Requirements	
Method Used to Determine the Highest Qualified Concentration	Empirical
Concentrations Above the Highest Qualified Concentration Fail	Yes
Include Unqualified Concentrations on Curve	Yes

Plate Layout Name	Number of Plates
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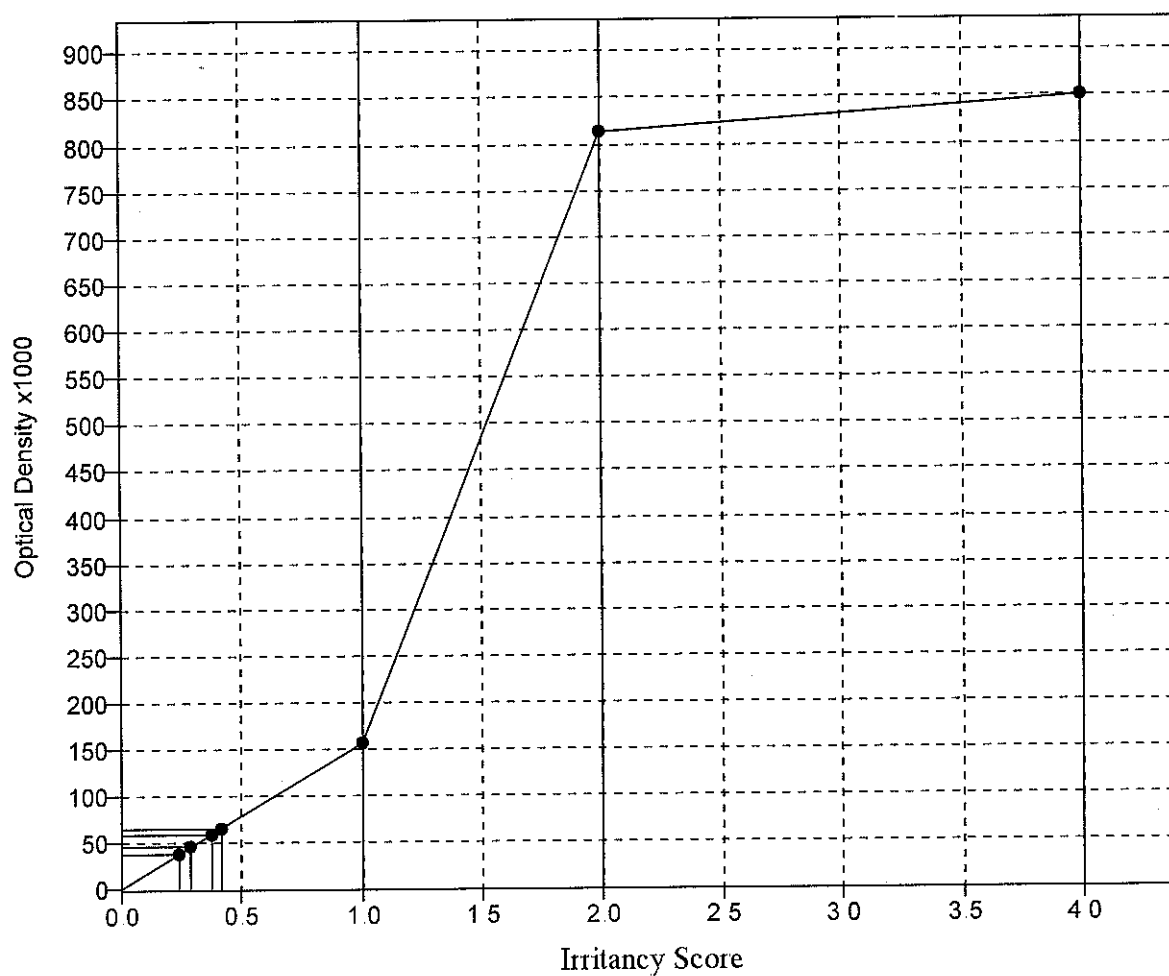
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CALIBRATION CURVE - ORIGINAL

Sample Description : Boswellin CG
 Sample Number : 31
 Assay Method : Dermal
 Protocol : Cosmetic - ID 052604
 Date : 03/19/05
 Technician : Sushmitha

Designation	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification
Cal 0	59				
Cal 1	156				
Cal 2	813				
Cal 3	850				
50 mg	55	-4	59	0.38	Non-Irritant
75 mg	57	-8	65	0.42	Non-Irritant
100 mg	42	-4	46	0.29	Non-Irritant
125 mg	35	-3	38	0.24	Non-Irritant

Calibration Curve



DOSE RESPONSE CURVE - ORIGINAL

Sample Description : Boswellin CG
Sample Number : 31
Assay Method : Dermal
Protocol : Cosmetic - ID 052604
Date : 03/19/05
Technician : Sushmitha

Designation	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification
Cal 0	59				
Cal 1	156				
Cal 2	813				
Cal 3	850				
50 mg	55	-4	59	0.38	Non-Irritant
75 mg	57	-8	65	0.42	Non-Irritant
100 mg	42	-4	46	0.29	Non-Irritant
125 mg	35	-3	38	0.24	Non-Irritant

Dose Response Curve

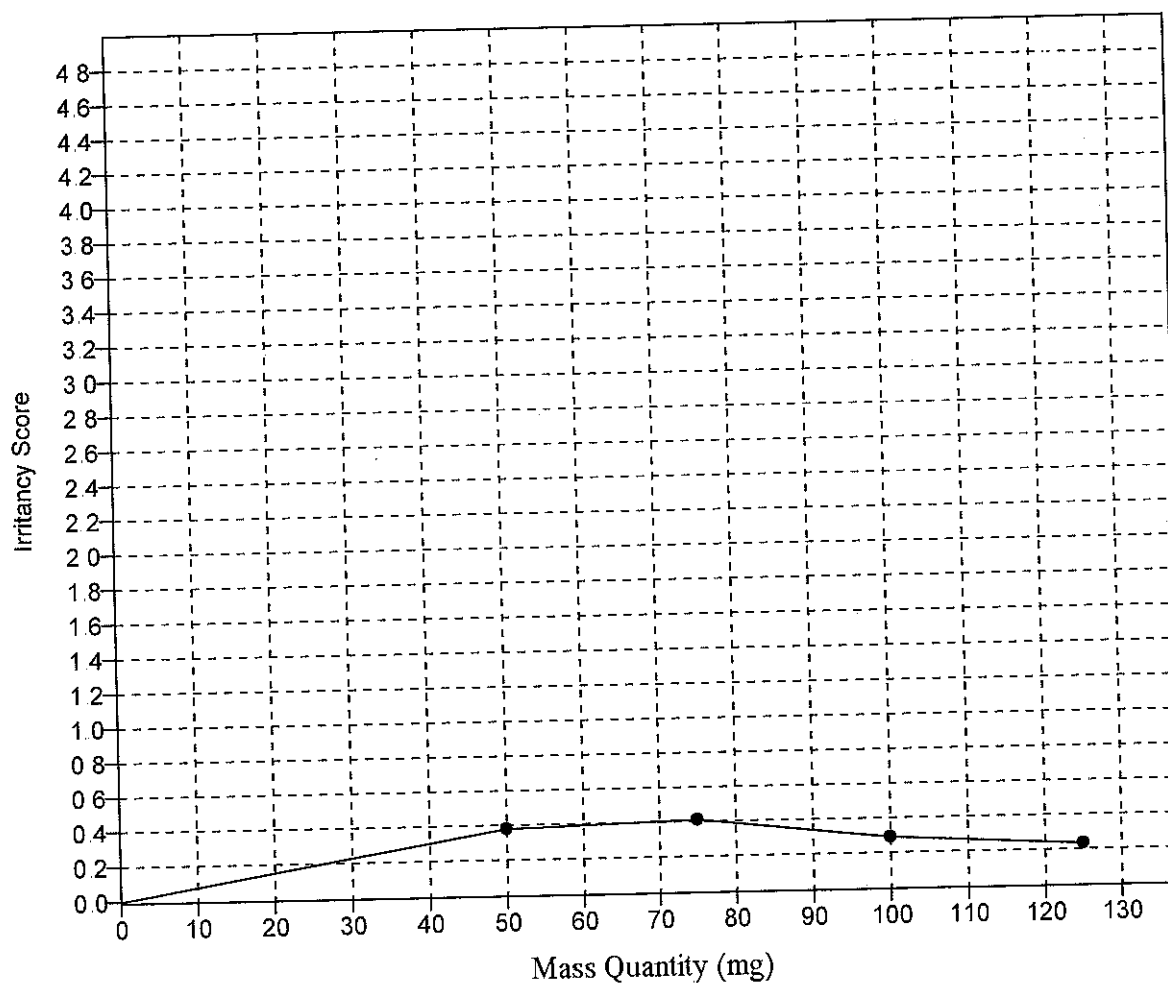


PLATE DATA - ORIGINAL

Sample Description: Boswellin CG
 Sample Number : 31
 Assay Method : Dermal
 Protocol : Cosmetic - ID 052604
 Date : 03/19/05
 Technician : Sushmitha

Blank Value : 55

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	-4	114	670	1912	70	1011	102	813	27	256	-11
B	-13	-13	-14	-14	-15	-14	-5	-12	-10	-13	-11	-13
C	59	-8	116	813	1911	109	818	134	851	35	286	909
D	-13	-12	-13	-14	-14	-14	-13	-14	-12	-10	-14	-13
E	215	-4	101	966	2699	120	893	165	736	38	311	127
F	-13	-13	-14	-14	-15	-14	-13	-14	-13	-14	-12	-13
G	872	-3	94	859	1966	157	687	144	908	43	238	144
H	-14	-14	-14	-15	-16	-15	-14	-14	-11	-13	-14	-14