Arginine Detection Report

Protocol No.: DET-PRO-016

Name of the product: Lumineux Oral Essentials clean and fresh

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Approved by: Nour Abochama Date: 2019-03-01

Nour Abochama
Director QA/QC, American Testing Lab Inc.

Approved by: Date: 3/1/2019

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1 Objective:

To determine the effect of Lumineux Oral Essentials clean and fresh on Arginine concentrations.

2 Scope:

A 3T3 clone A31 Cell Line from mouse was exposed to a 5000 µg/ml solution of test product for 24 hours. A determination of Arginine concentration was performed using HPLC method.

3 Responsibility:

The persons responsible for the process will perform the and record the information. The responsible person will supervise the study, verify the completion of the records and write the report. Quality Assurance will review and approve the Report.

Name of the Personnel	Designation	Signature
__Nour Abochama_____	__Director QA/QC___	Nour Abochama _____
__Reem Samman_____	___Chemist_____	ras _____

4 Materials, Equipments and documents:

List with Equipment No. and all Standard Operation Procedures (SOPs) for the normal processes under test.

4.1 List of Equipment:

Name of Equipment	Equipment No.
HPLC with UV detector	LAB-2426
Balance	LAB-2233
Pipette	LAB-2422
Incubator	LAB-2223
HPLC Column, Luna C18, 250 x 4.6 mm, 5µm	LAB-0175

Complied By: ras **Date:** 2019-02-28

Reviewed By: Nour Abochama **Date:** 2019-02-28

5 Analytical Method

5.1 Standers and Reagents:

5.1.1 L-Arginine Reference Standard (or equivalent);
5.1.2 Phosphoric Acid H_3PO_4, ACS grade (or equivalent);
5.1.3 Acetonitrile HPLC grade (or equivalent)
5.1.4 Purified water USP grade (or equivalent);

5.2 Solutions:

5.2.1 *Mobile Phase:* 90% of Line A: 10% of Line B where: Line A: 0.1% Phosphoric Acid, Line B: Acetonitrile.

5.3 standard:

5.3.1 Accurately weight and transfer about 13 mg ± 1.0 mg of L-Arginine Reference Standard into 10 mL volumetric flask.
5.3.2 Dissolve in about to ¾ of volume with purified water.
5.3.3 Complete to volume with mobile phase.
5.3.4 Mix well.

5.4 Sample:

5.4.1 Transfer 1 ml of test sample into 10 mL volumetric flask.
5.4.2 Dissolve in about to ¾ of volume with purified water.
5.4.3 Swirl to homogenize the solution.
5.4.4 Let the solution to cool at room temperature.
5.4.5 Complete to volume with purified water and mix well.
5.4.6 Immediately filter through a 0.45 µm nylon filter and transfer into HPLC vial discarding the first 2 mL.

5.5 Chromatographic Conditions:

5.5.1	Injection Volume:	5 µL;
5.5.2	Wavelength:	195 nm;
5.5.3	Mobile Phase:	As per section 5.2.1 of this method;
5.5.4	Flow Rate:	0.5 mL/min;
5.5.5	Expected RT:	3.7 min;
5.5.6	Run time:	15 min.

5.6 Injection Run:

5.6.1 1 inj Mobile Phase BLK;
5.6.2 5 inj working standard STD;
5.6.3 1 inj samples;
5.6.4 1 inj control (working standard) QSTD

5.7 System Suitability:

5.7.1 From the 6 injections of standard, area RSD should not be more than 5.0%.
5.7.2 Recovery between standard control and mean area of standard should be within 95.0%-105.0%.

5.8 Calculations:

5.8.1 Calculate against the mean area of first 5 injection of standard.

5.8.1.1 Recovery standard

$$\textbf{\textit{\% Recovery =}} (\textbf{\textit{Area}}_{qstd}\textbf{\textit{/Mean Area}}_{std})\textbf{\textit{×100}}$$

Where:

$Area_{qstd}$	=	Area of peak of interest in standard or Control.
Mean $area_{std}$	=	Mean area of peak of interest in standard

5.8.1.2 Sample Calculation

$$\%\ \text{of Arginine in Products} = (Area_{spl} / \text{Mean } Area_{std}) \times (C_{std}/W_{spl}) \times 100\ mL \times 100$$

Where:

$Area_{spl}$	=	Area of Arginine in Sample preparation
Mean $Area_{std}$	=	Mean Area of Arginine in working standard preparation
C_{std}	=	Concentration of standard in working standard (mg/mL)

W_{spl} = Weight of sample (mg)

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6 Protocol

6.1 The test item is prepared y adding 10% (v/v) FCS and 1% antibiotics (v/v) to the artificial Sylvia
6.2 Prepare 5000 µg/mL solution of Lumineux Oral Essentials clean and fresh . Expose the test
6.3 item to the product solution for 24, 36, 48, 60, 72, 84 and 96 hours then prepare as described in section 5.4.

7 Approval:

7.1 Submit the Document to QA for review and approval.

8 Results:

Exposure Time	Blank (µg/ml)	Test Product (µg/ml)	Water (µg/ml)	Ref. Product (µg/ml)
0 hours	0.00	0.2116	0.00	0.223
24 hours	0.00	0.1692	0.00	0.222
36 hours	0.00	0.1269	0.00	0.223
48 hours	0.00	0.1058	0.00	0.221
60 hours	0.00	0.0846	0.00	0.02
72 hours	0.00	0.0423	0.00	0.02
84 hours	0.00	0.0212	0.00	0.02
96 hours	0.00	0.0211	0.00	0.02

9 Conclusion:

The Arginine-specific gingipain (Rgp) concentration have been 99% decreased in test item after 96 hours of exposure.
The solution at 5000 µg/mL is prepared form Lumineux Oral Essentials clean and fresh Lot# 17K13B2

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Nour Abochama, M. Sc.,