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Fitodenta UAB
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LITAUEN

Person in charge Ms A. Kordan - 6872
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Report date 23.01.2024

Analytical report PR-24-JR-000062-01

Sample Code 799-2023-00026679

Reference	Fitodenta Oromuscosal Relief Spray Forte
Client sample code	1
Purchase order code	N/A
Lot-no.	02/2023-1ORF
Number of received Samples	3
Ordered by	Ms. Evelina Marcevičienė
Submitted by	Ms. Evelina Marcevičienė
Carrier	UPS
Reception date	21.11.2023
Start/end of analyses	22.11.2023 / 23.01.2024

TEST RESULTS

00000 Minimal Inhibitory Concentration (MIC)
Subcontracted to a Eurofins laboratory

Minimal Inhibitory Concentration (MIC)	see attachment

Note: The report contains the preliminary results.

Signature _____
Teamlead ASM Cosmetic CPT Hamburg (Adelina Kordan)

Test Report STULV23AA3651-1

SPONSOR	Eurofins Consumer Product Testing GmbH Am Neulaender Gewerbepark 4 DE - 21079 Hamburg Germany		
TEST METHOD	- CLSI M45 Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria - CLSI M07 - Methods for Dilution Antimicrobial susceptibility test for bacteria that growth Aerobically; Approved Guideline – 11th, Ed.		
TEST ITEM			
Matrix of the product	Cosmetics		
PRODUCT NAME	OROMUCOSAL RELIEF SPRAY		
ACTIVE INGREDIENT	Not Provided		
STORAGE	Room Temperature		
HAZARD INFORMATION	Not Provided		
ANALYZED SAMPLE			
BATCH	02/2023-1ORF		
CODE	Not Provided		
MANUFACTURING DATE	Not Provided		
EXPIRY DATE	Not Provided		
PARCEL REGISTRATION NUMBER	IP-LV-2023327-ALI		
RECEIVING DATE	23/11/2023		
MATERIAL ITEM ALIQUOT	LV-MAT-PBEP-23-340-0D57:a		
ANALYSIS STARTING DATE	09-Jan-2023	**ANALYSIS ENDING DATE**	12-Jan-2023

EXPERIMENTAL DESIGN	
Minimal Inhibitory Concentration (MIC) is defined as the highest dilution (or the lowest concentration) of antimicrobial agent that inhibits the growth of test microorganism after 24 h under a standardized set of conditions. On the test items, a study has been performed in order to define the Minimal Inhibitory Concentration (MIC), against Staphylococcus aureus ATCC6538, as Sponsor's request. Study performed consist of a test that combines a disk diffusion pattern with the determination of Minimum Inhibitory Concentration (MIC). Plate of culture of bacterial suspension on Mueller Hinton Agar (MHA) has been prepared, by using the sterile cotton swab; then filter paper discs of about 6 mm in diameter was added with 50 µl of test item dilutions and then put on to the inoculated agar plate then incubated at 37±1°C for 24 hours. After incubation, bacterial growth becomes visible on the plate, and inhibition halo is seen at inhibitory concentration. Test has been done in three independent replicas, twice each.	
EXPERIMENTAL CONDITIONS	
The Test Item has been tested neat and at eleven serial two-fold dilutions, according to CLSI. Minimal Inhibitory Concentration (MIC) was performed by agar diffusion method.	
TEST STRAINS	*Staphylococcus aureus* ATCC6538
REAGENTS	The validity of media and reagents have been verified according to Internal procedure. – Buffered Peptone water (BPW) – Tryptone Soy Broth (TSB) – Muller Hinton Agar Calcium Adjusted (MHBCA) – Dimethyl sulfoxide (DMSO) – Tryptone Soy Agar (TSA) – filter paper discs about 6 mm in diameter
EQUIPMENT	The validity of instruments and equipment has been assured following internal procedures before starting the analyses. Standard microbiology laboratory equipment has been used: – Laminar flow filtered work area – Micropipettes – Thermostat at 37±1°C

ASSAY	**Inoculum preparation** Day prior the test, fresh culture of bacterial strain has been grown on TSA slant at 37±1°C for 24 hours. Five well-isolated colonies from the agar plate culture have been transferred into tryptic soy broth (TSB) tube then incubated at 37±1°C for 2-6 h to achieves a suspension approximately 1-2 × 10^8 cfu/mL **Assay sample preparation** The Test Item has been tested neat and at eleven serial two-fold dilutions in DMSO, according to CLSI **Inoculation** Two plates (test in twice) using swab impregnated with the inoculum suspension 90 mm plates have been streaked on the entire agar surface three times, rotating the plate 60 degrees each time to evenly distribute the inoculum. Plates have been allowed dry for approximately 15 to 20 minutes. **Assay** Test has been performed in three independent replicates. Each replicate has been performed in twice. Plates have been incubated for 24 hours at 35±1°C. Following incubation, the zones of inhibition has been observed and recorded. **ASSAY VALIDITY CRITERIA** Assay validity criteria were satisfied when: Positive Control shows homogeneous growth. Negative Control (diluent) doesn't show growth. Negative Control (Test Item dilution) doesn't show growth.
CALCULATION	***Calculation of the viable count (cfu/g)*** The viable microorganism concentration in inoculum has been calculated applying the following formula: $$N(cfu/ml) = \frac{c}{(n_1 + 0.1n_2)d}$$ where: c = sum of colonies counted on all countable plates n_1 = number of counted plates in the lower dilution n_2 = number of counted plates in the higher dilution d = dilution factor corresponding to the lower dilution The counting was performed using the number of colonies counted on Petri plates.
INTERPRETATION OF RESULTS	The highest dilution (or the lowest concentration) of Test Item that inhibits the growth of test microorganism after 24 h under a standardized set of conditions is considered the MIC value.

RESULTS

Assay validity Criteria was satisfied.

Preparation of assay sample						RESULT					
Tube ID	Df	Tube Volume (ml)	Source	Added to	DMSO Volume (ml)	I Replicate		II Replicate		III Replicate	
						Inoculum (cfu/ml)					
						1.2×10^8		1.5×10^8		1.1×10^8	
						Serie I	Serie II	Serie I	Serie II	Serie I	Serie II
1	1	15,0	1	+	0	-	-	-	-	-	-
2	2	10,0	1	+	10	-	-	-	-	-	-
3	4	5,0	1	+	15	-	-	-	-	-	-
4	8	2,5	1	+	17,5	-	-	-	-	-	-
5	16	10,0	8	+	10	+	+	+	+	+	+
6	32	5,0	8	+	15	+	+	+	+	+	+
7	64	2,5	8	+	17,5	+	+	+	+	+	+
8	128	10,0	64	+	10	+	+	+	+	+	+
9	256	5,0	64	+	15	+	+	+	+	+	+
10	512	2,5	64	+	17,5	+	+	+	+	+	+
11	1024	10,0	512	+	10	+	+	+	+	+	+
12	2048	5,0	512	+	15	+	+	+	+	+	+

Legenda:
Df: Dilution factor
-: no growth (Inhibition)
+: growth (no inhibition)

CONCLUSIONS

On the basis of obtained results, interpreted according to CLSI M45, can be stated that Test Item "OROMUCOSAL RELIEF SPRAY" has antimicrobial activity defined as Minimal Inhibitory Concentration (MIC) until a 1:8 dilution factor against *Staphylococcus aureus*, in experimental condition adopted.

ADDENDA

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