2.5 Finished Product Technical Specifications & Method of Analysis

Finished Product Specification:

S.No	TEST	SPECIFICATION
1.	Description	Clear colorless solution
2.	Identification Test by GCMS	Nitric Oxide (NO) peak with molecular weight (30m/z) should appear in GC-MS chromatogram.
3.	pH at 25°C	Between 2.50 – 5.00
4.	Volume in container	The volume should not be less than 15mL from each bottle of the container
5.	Assay: Each mL of solution contains	
	Sodium Nitrite	80.0% - 120.0% of the labeled amount (4.00 mg/mL – 6.00 mg/mL) of Sodium Nitrite
	Benzalkonium Chloride	80.0% - 120.0% of the labeled amount (0.16 mg/mL – 0.24 mg/mL) of Benzalkonium chloride
6.	Microbial Limit Test	Complies

Shelf life specification:

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6.	Microbial Limit Test	Complies

Method of Analysis of Finished Product:

1.0 DESCRIPTION:

Inspect the sample visually for its appearance and record its findings.

2.0 IDENTIFICATION TEST:

A. Identification of Nitric Oxide by GCMS

S.No.	Instruments	Make
1	Semi Microbalance	Sartorius or equivalent
2	GCMS	Shimadzu or equivalent

Chromatographic Conditions:

Column	:	Agilent VF 624MS 30m x 250µm x 1.40 µm		
Type	:	VF - 624 ms		
Carrier Gas	:	Helium		
Injector Temperature	:	40°C		
Flow	:	1.0mL/min		
Split	:	on		
Split ratio	:	5:1		
Oven Programme:				
Initial temperature	:	40°C		
Hold Time	:	1.0min		
Programme rate 1	:	20.0°C/min → 240°C → 2min		
Detector Parameters:				
Type	:	MSD		
Source Temp	:	250°C		
Interface temperature	:	230°C		
Acquisition Type	:	Q3 Scan;	Q3 SIM	
Ch1 m/z	:	25.00	500.00	30.00
Solvent cut time	:	0.0min		
End Time	:	13.0min		
Head Space Parameters:				
Oven Temperature	:	60°C		
Loop/Injector temperature	:	100°C		
Transfer line temperature	:	100°C		
Vial equilibration Time	:	15.0min		
Pressurization Time	:	0.20min		
Pressurization equilibration Time	:	0.20min		
Loop/injector fill time	:	0.20min		
Loop/injector equilibrator time	:	0.05min		
Injection Time	:	1.0min		
Injection volume	:	NA		

GC Cycle time	:	25.0min
Head space vial size	:	20.0mL

Preparation of Sample:

Spray the sample into a head space vial and crimp the vial immediately.

Note: Nitric Oxide (NO) peak with molecular weight (30m/z) should appear in GC-MS chromatogram.

3.0 pH at 25°C:

Transfer sufficient quantity of sample into a clean and dry beaker and record the pH with suitable calibrated pH meter (Mix the contents of both the bottles and check the pH).

4.0 VOLUME IN CONTAINER

Select 2 containers, shake the contents of 2 containers individually.

Procedure

Carefully discharge the contents of each container into separate dry graduated cylinders. Transfer the sample contents into the graduated cylinder, should be free from bubbles, measure the volume of each mixture.

Acceptance criteria:

The volume of each container is not less than the nominal volume.

5.0 ASSAY

C. ASSAY of SODIUM NITRITE (By Titration):

Instruments:

S.No.	Instruments	Make
1	Semi Microbalance	Sartorius or equivalent

Chemicals/Reagents:

S.No.	Chemicals/Reagents	Make	Grade
1	Potassium permanganate	Merck or equivalent	AR or equivalent
2	Sulfuric Acid	Merck or equivalent	AR or equivalent
3	Oxalic Acid	Merck or equivalent	AR or equivalent

Sample solution:

Nominally 50mg of Sodium Nitrite from an accurately measured volume of Nasal Spray.

Titrimetric System:

Mode: Direct Titration

Titrant: 0.1N Potassium permanganate

Endpoint Detection: pink colour

Procedure:

Accurately transfer about 10 mL of test sample (equivalent to 50mg of Sodium Nitrite) in to a beaker containing a mixture of 50mL of 0.1N potassium permanganate,100mL of water,and 5mL of sulfuric acid. When adding the sodium nitrite solution, immerse the tip of the pipet beneath the surface of the permanganate mixture. Warm the liquid to 40°,allow it to stand for 5minutes,and add 25mL of Oxalic acid. Heat the mixture to about 80° and titrate with 0.1N potassium permanganate. Each mL of 0.1N potassium permangante is equivalent to 3.450 mg of $NaNO_2$.

Note: Determine the blank titration without test sample.

Calculation:

$$\text{Assay by Titration} \quad \frac{\text{T.V x N x 3.450 x 100}}{\text{SPL Volume x LC}}$$

Titer value (T.V) = Sample titer volume-blank titer volume
N= Normality of Potassium permanganate
LC= Label claim of Sodium nitrite (5mg)

D. ASSAY of BENZALKONIUM CHLORIDE (By HPLC):

Instruments:

S.No.	Instruments	Make
1	Semi micro balance	Sartorius or equivalent
2	HPLC	Agilent 1200 series or equivalent
3	pH meter	Thermo Orion or equivalent

Chemicals/Reagents:

S.No.	Chemicals/Reagents	Make	Grade
1	Perchloric acid (about 70%)	ACS or equivalent	HPLC or equivalent
2	Sodium perchlorate	ACROS or equivalent	ACS or equivalent

S.No.	Chemicals/Reagents	Make	Grade
3	Methanol	Merck or equivalent	HPLC or equivalent
4	Acetonitrile	Merck or equivalent	HPLC or equivalent
5	Water	Siemens or equivalent	HPLC or equivalent

Chromatographic conditions:

Column	: Waters X-Bridge C18, 250mm x 4.6mm, 5µm
Flow rate	: 1.5 mL/min
Injection volume	: 50µL
Column oven temperature	: 25°C
Sample cooler temperature	: 25°C
Wavelength	: 210 nm
Runtime	: 30 minutes
Elution mode	: Isocratic

Preparation of diluted Perchloric acid:
Transfer 1 mL of perchloric acid into 100 mL of water and mix well.

Preparation of Buffer:
Weigh and transfer 14.0 g of sodium perchlorate into 1000 mL of water and mix well. Then adjust the pH of the solution to 4.50 ± 0.05 with diluted perchloric acid and mix well.

Preparation of Mobile Phase:
Prepare a degassed mixture of buffer and acetonitrile in the ratio of 30:70 v/v respectively. Sonicate for 5 minutes to degas.

Diluent:
Use mobile phase as a diluent

Preparation of Standard Stock Solution: (0.2 mg/mL)
Accurately weigh and transfer 40 mg of benzalkonium chloride working/ reference standard (50% Benzalkonium chloride) into 100 mL volumetric flask, add 70 mL of methanol, sonicate to dissolve, make up to the volume with methanol and mix well.

Preparation of Sample Solution: (0.2 mg/mL)
Directly spray the sample approximately 1-1.5mL in a HPLC vial and inject.

Procedure:

Before analysis equilibrate the chromatographic system with mobile phase until stable baseline is obtained. Separately inject 50 µL of diluent, standard solution and sample solutions into HPLC.

Analyte Name (BKC)	Retention time (mins)
C12 homologue	~7.8
C14 homologue	~15.7

Sequence of Injections:
1 x Blank
5 x Standard solution
2 x Sample solution
1 x Bracketing standard solution
Note: End run with standard solution.

Acceptance Criteria:

iv. The relative standard deviation for sum of areas of benzalkonium homologues (C12 & C14) peaks from five replicate injections of standard solution should be not more than 2.0%.
v. The tailing factor for each of benzalkonium homologues (C12 & C14) peaks in first injection of standard solution should be not more than 2.0
vi. The theoretical plates for each of benzalkonium homologues (C12 & C14) peaks in first injection of standard solution should be not less than 2000

Calculation: Calculate the % assay of benzalkonium chloride using the following formula.

$$\text{Benzalkonium chloride in mg/mL} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{3}{50} \times \frac{10}{SV} \times \frac{P}{100}$$

$$\%\ \text{Assay} = \frac{\text{Benzalkonium chloride in mg/mL}}{LA} \times 100$$

Where,

AT = Average sum of areas of benzalkonium homologues (C12 & C14) in the sample solution.
AS = Average sum of areas of benzalkonium homologues (C12 & C14) in the standard solution.
SV = Volume of the sample in mL
LA = Labeled amount of benzalkonium chloride per mL (0.1 mg).
P = Potency of benzalkonium chloride working standard in % (on as is basis)

6.0 MICROBIAL LIMIT TEST

Proceed as per the USP General chapter <71>

7.0 REFERENCES/ CROSS REFERENCES:

USP General chapters and USP Monograph of Sodium Nitrite.