APPROVALS:

	Name	Designation& Department	Signature and Date
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Approved by (SK Pharmatech Solutions)	Dr.K.Jagadeswara Rao	Director Technical	
Approved by Customer			

Customer Address:
Contact Person:

PURPOSE:

To perform the analysis of Polysorbate 80 NF in line with USP Monograph through the PO No. 9100028158 issued on 29 January 2025.

SCOPE:

This document is applicable for Polysorbate 80 by using following testing parameters.

S.No.	Test parameter
1	Description
2	Solubility
3	Identification by IR
4	Residue on ignition (<281>)
5	Specific Gravity (<841>)
6	Viscosity (<911>)
7	Fats and Fixed Oils-Hydroxyl value (<401>)
8	Fats and Fixed Oils-Acid value (<401>)
9	Fats and Fixed Oils-Peroxide value (<401>)
10	Fats and Fixed Oils-Saponification value (<401>)
11	Water determination (<921>)
12	Ethylene Oxide and Dioxane
13	Assay-Composition of Fatty acids

RESPONSIBILITY:

This report shall be prepared by Head-AD/Designee from SK Pharmatech Solutions or designee.

This report shall be reviewed by Head-QA/Designee from SK Pharmatech Solutions or designee.

This report shall be reviewed by Head-CRD/Designee from SK Pharmatech Solutions or designee.

This report shall be approved by Director-Technical from SK Pharmatech Solutions.

This report shall be approved by Customer.

MATERIAL DETAILS:

Material name : Polysorbate 80

Supplier Batch No. : 2179038

CAS number : 9005-65-6

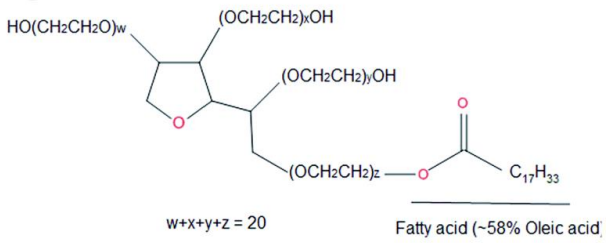
Appearance : Lemon to amber-colored, oily liquid

Molecular weight : NA

Molecular formula : NA

Solubility : Very soluble in water, soluble in alcohol and ethyl acetate and insoluble in Mineral oil

Chemical Structure:



IUPAC Name: Polyoxyethylene 20 sorbitan monooleate
Storage : Store in an air tight container, protected from light.

Test Parameters:

1. **Description:**

   Procedure: Taken 5 mL of sample into a test tube and visually observed the sample.

   Observation: Lemon coloured, oily liquid

   Refer documentation: Page No.01

2. **Solubility:**

A. Procedure:

Taken 1.0140 g of sample in 1ml water and visually observed.

Observation: Very soluble in water.

B. Procedure:

Taken 1.0031 g of sample in 30ml alcohol and visually observed.

Observation: Soluble in alcohol.

C. Procedure:

Taken 1.0 g of sample in 30ml ethyl acetate and visually observed.

Observation: Soluble in ethyl acetate.

Refer documentation: Page No.01

D. Procedure:

Taken 1.0 g of sample in 30ml mineral oil and visually observed.

Observation: Insoluble in mineral oil.

Refer documentation: Page No.01

3. Identification:

A. It meets the requirements of the test for **Composition of Fatty Acids.**

B. It meets the requirements for **Infrared Spectroscopy-Refer Annexure-I**

4. Residue on Ignition (<281>):

Procedure:

Wt. of empty crucible W_1 = 92.0146 g

Wt. of crucible + Sample W_2 = 94.0257 g

Wt. of crucible after ignition W_3 = 92.0184 g

Calculation:

$$\frac{W_3-W_1}{W_2-W_1} \times 100$$

$$= \frac{92.0184 - 92.0146}{92.0146 - 92.0146} \times 100$$

$$= \frac{0.0038}{2.0111} \times 100$$

$$= 0.188\ \%$$

Result: 0.19%

Refer documentation: Page No.08

5. Specific Gravity (<841>):

Procedure:

Wt. of empty specific gravity bottle W= 21.8292g

Wt. of sample W_1= 24.8153g

Wt. of water W_2 = 24.6185g

Wt. of water at 25^0C = 0.9970g

Calculation:

$$W_1\text{-}W \,/\, W_2\text{-}W$$

$$= 24.8153\text{-}21.8292 \,/\, 24.6185\text{-}21.8292$$

$$= 2.9861/2.7893 \times 0.9970$$

$$= 1.07$$

Result: 1.07

Refer documentation: Page No. 09

6. Viscosity-Capillary method (<911>):

Procedure:

The capillary Ostwald's type viscometer (SKP-EQP-OCV-042) is filled with the sample upto the above level of the upper mark and immersed in water and maintained in a vertical position for time period of NLT 30 minutes to allow the sample temperature to reach equilibrium, recorded the time in seconds required for the liquid to flow from the upper mark to the lower mark.
Start time: 11:40

End time: 15:40

Total time: 4 hours

Calculation:

$K = \eta (p \times t)$

η = known viscosity of the liquid

p = density of the liquid

t = flow time for the liquid to pass from the upper mark to the lower mark.

= 500 / (1.06 x 14,400)

= 500 / 1526.4

= 0.03

V = k x t

= 0.03 x 14,400

= 432 centistokes

Result: 432 centistokes

Refer documentation: Page No.10-13

7. Fats and Fixed Oils – Hydroxyl value (<401>):

Procedure:
Preparation of 0.5 N of Alcoholic Potassium Hydroxide:

Taken 8.5g of Potassium Hydroxide into 250 mL of alcohol and added 240mL of water and mixed well.

Taken 1.0124g of sample into 250mL of conical flask and added 5.0mL of pyridine- acetic anhydride reagent (9ml of pyridine & 3 ml of acetic anhydride). Taken 5.0 mL of pyridine- acetic anhydride reagent into another 250ml conical flask as reagent blank. Fitted with glass-joined reflux condensers, heated on stream bath for 1 hr added 10 mL water through each cinders is heated on stream bath for 10min more, cooled & Added 25mL butyl alcohol (Previously Neutralized to phenapthaline TS) & titrated with 0.5 N of alcoholic potassium hydroxide.

Calculation: [(M x N) / W] × (B-T) + Acid Value

= [(56.11 x 0.49) / 2.0124] x (39.5 – 34.5) + 1.08

= [(37.4939/2.0124)] x 5.0 + 1.08

= 68.311+ 1.08

= 69.4

Result: 69.9

Refer documentation: Page No. 14-15

8. Fats and Fixed Oils-Acid Value (<401>):

Procedure:

Weighed 5.0012g of sample into 50ml of a mixture of equal volumes of alcohol and light petroleum (previously neutralized with 0.1N sodium hydroxide) using 0.5ml phenolphthalein TS, titrated with 0.1N sodium hydroxide to the end point pink colour.

Calculation:

(M x V) (N/W)

M = Molecular weight of potassium hydroxide

V = Volume of 0.1N sodium hydroxide VS consumed (mL)

N = Normality of sodium hydroxide VS

W = Weight of the sample taken (g)

= (40 x 1.5) (0.09/5.0012)

= 60 x 0.018

= 1.08

Result: 1.08

Refer documentation: Page No.16

9. Peroxide Value (<401>):

Procedure:

Preparation of 0.01M Sodium thiosulphate:

Dissolved 0.520 g of sodium thiosulphate in 200 mL of water, mixed well.

Preparation of saturated potassium iodide solution:

Taken potassium iodide into a beaker and added 10 mL of water and sonicated to dissolve. Added potassium iodide until the solution appears with un-dissolved crystals.

Sample Preparation:

Transferred the sample 10.0077g into a beaker and dissolved with 20 mL of acetic acid. Added 1 mL of saturated potassium iodide solution, kept on bench top for 1 min. Added 50 mL of water and titrated with 0.01M Sodium thiosulphate and determined the end point potentiometrically.

Calculation:

Peroxide value = 1000 (Vt-Vb) *M/W

Where,

Vt= Volume of sodium thiosulphate consumed for sample

Vb= Volume of sodium thiosulphate consumed for blank

M= Molarity of Sodium thiosulphate

W= Weight of the sample

Results= Volume of thiosulphate consumed for blank: 0.960 mL

Volume of sodium thiosulphate consumed for sample: 8.743 mL

=1000 (8.743-0.960)*0.01/10.0077

= 1000 (7.783) * 0.01/10.0077

= 77.83/10.0077

= 7.77

Refer documentation: Page No. 17-20

10. Fats and Fixed Oils - Saponification Value (<401>):

Preparation of 0.5 N Alcoholic Potassium Hydroxide solution:
Taken 3.017 g of KOH and added 2.5 mLwater and add 100 mL of ethanol then mixed well.

Preparation of 0.5 N Hydrochloric Acid VS:
Taken 100 mL of volumetric flask and added 50 mL water and add 4.25 mL of HCL and made up with water upto the mark and mixed well.

Standardization of 0.5 N HCL VS:
Taken 1.5007 g of sodium carbonate and added 100 mL water and added 0.1 mL of methyl red solution then titrate with 0.5 N of HCL to pink color.

Preparation of Blank:
To a 250 mL glass flask fitted with a reflux condenser, added 30 mL 0.5N alcoholic KOH, attached the condenser and heated under reflux for 60 minutes, remove the solution, cooled and added 1.0 mL phenolphthalein TS and titrated with 0.5 N of HCL VS solution to the end point.

Preparation of Sample:
Taken 4.0138g of sample into a 250 mL glass flask fitted with a reflux condenser, added 30 mL 0.5N alcoholic KOH, attached the condenser and heated under reflux for 60 minutes, remove the solution, cooled, added 1.0 mL phenolphthalein TS and titrated with 0.5 N of HCL VS solution to the end point.
Consumed Volume of 0.5 N HCL = 20.9

Calculation: [Mr x (Vb-VT) x N] / W

M = Molecular wt. of potassium hydroxide
Vb = Volume of 0.5 N HCL Consume in the Blank
VT = Volume of 0.5 N HCL Consume in the Test
N = Normality of HCL
W = Weight of the Sample (Test)
= [56.11 X (31-24) X 0.5] / 4.0138
= 56.11 X 7 X 0.5 / 4.0138
= 48.9

Result: 48.9

Refer documentation: Page No. 21-22

11. Water Determination (<921>):

Taken 1.2180g of sample into KF titration vessel and determined the water content of sample by using Karl Fisher Apparatus.

Result: 2.23%

Refer documentation: Page No. 23

12. Ethylene Oxide and Dioxane:

S.No.	Name of the standard	Batch No.
1	Dioxane	SK-WS-22-096
2	Ethylene oxide	984473/CPA Chem

Preparation of ethylene oxide standard solution:

Transferred 0.5 mL of ethylene oxide into a 50 mL volumetric flask and diluted to volume with water and mixed well.

Pipetted out 1 mL of above ethylene oxide standard stock solution into a 250 mL volumetric flask and diluted to volume with diluent and missed well.

Preparation of Dioxane standard solution:

Transferred 0.5 mL of dioxane into a 500 mL volumetric flask, added 250 mL of water and mixed well, diluted to volume with water and mixed well.

Preparation of Acetaldehyde standard solution:

Weighed and transferred 10 mg of acetaldehyde into a 1000 mL volumetric flask containing 500 mL of water. Sonicated to dissolve and diluted to volume with water and mixed well.

Preparation of standard solution:

Taken 6.0 mL of ethylene oxide standard solution, 2.5 mL of dioxane standard solution into a 25 mL volumetric flask and diluted to volume with water and mixed well.

Preparation of reference solution:

Transferred 2 mL of Acetaldehyde standard solution and 2 mL of ethylene oxide standard solution into a 10 mL headspace vial and sealed the cap with Teflon cap and aluminium seal with a crimper.

Preparation of sample solution-A:

Weighed and transferred 1.0 g of polysorbate 80 sample into a 10 mL head space vial and added 2 mL of water and sealed with Teflon seal and aluminium cap with crimper.

Preparation of sample solution-B:

Weighed and transferred 1.0 g of polysorbate 80 sample into a 10 mL head space vial, added 2 mL of standard solution and 2 mL of water and sealed with Teflon seal and aluminium cap with crimper.

Chromatographic conditions

GC parameters:

Column	: IC-5, 0.53-mm × 50-m x film 5µm. (Cat No. 1512-5350-50)
Column ID	: SK-GC-008
Brand Name	: Inert chrom
Oven temperature	: Initially maintain at 70.0 ° C and then rise to 250°C at the rate of 10.0°C /min, and hold for 5 minutes.
Column flow	: 4 ml / min
Mode	: Constant Pressure
Detector Gas	: Air (350 ml/min)
Hydrogen	: 35 ml/min
Carrier Gas	: Helium
Make up Gas flow	: 30 ml/min
Septum flow	: 3.0 ml/min
Split ratio	: 1:3.5
Injection volume	: 1 µL
Injector Temperature	: 85ºC
Detector Temperature	: 250ºC
Equilibration time	: 1 µL
GC cycle time	: 35 min

Head Space parameters:

Oven temperature	: 80 ºC
Needle temperature	: 82 ºC
Transfer line temperature	: 85 ºC
Pressurization time	: 1.0 min
Equilibration time	: 30 min
Cycle time	: 35 min
Withdrawal time	: 0.2 min

System suitability:

Inject Blank (water), reference solution, sample solution-A, sample solution-B into the GCHS and calculate the content of ethylene oxide content and dioxane content with the following calculation.

Content of Ethylene oxide:

= (2 x Ceo x Aa) / (Ab-Aa)

Where,

Ceo= Concentration of ethylene oxide in sample solution B

Aa= Peak area of thylene oxide from sample solution-A

Ab= Peak area of thylene oxide from sample solution-B

Result: = (2 x 0.002 x 280.76) /(250.13-280.76)

= 1.12304/30.63

= 0.04 ppm

Content of Dioxane:

= (2 x D x Cd x Aa) x 1000/ (Ab-Aa)

Where,

D= Density of dioxane (1.03g/mL)

Cd= Concentration of dioxane in sample solution B

Aa= Peak area of dioxane from sample solution-A

Ab= Peak area of dioxane from sample solution-B

System suitability:

Result: Not detected

Refer documentation: Page No. 25-33

13. Assay-Composition of Fatty Acids:

S.No	Name of the Fatty acid	Batch No.	Make
1	Methyl myristate/ **Methyl Tetradecanoate Standard**	SK-WS-24-258	SKPHARMATECH
2	Methyl palmitate Standard	SK-WS-25-526	SKPHARMATECH
3	Methyl Stearate Standard	SK-WS-25-525	SKPHARMATECH
4	Methyl Arachidate Standard	SK-WS-22-058	SKPHARMATECH
5	Methyl Oleate Standard	SK-WS-25-524	SKPHARMATECH
6	Methyl eicosenoate/ **Methyl cis-11-Eicosenoate**	SK-WS-25-545	SKPHARMATECH
7	Methyl Behenate Standard	SK-WS-25-527	SKPHARMATECH
8	Methyl lignocerate	SK-WS-22-057	SKPHARMATECH
9	FAME Standard Mixture for Polysorbate	SK-PS-25-1454	SKPHARMATECH

Preparation of diluent:

Weighed and transferred 0.5g of sodium hydroxide into a 250 mL of volumetric flask. Add 100 mL of methanol and sonicate to dissolve. Dilute to volume with diluent and mixed well.

Preparation of boron trifloride solution:

Used commercially available Boron trifloride solution.

Preparation of saturated sodium chloride solution:

Weighed and dissolved sodium chloride in water until the solution gets saturated and no sodium chloride is dissolved.

Preparation of Reference solution-A:

Weighed and transferred 5 mg Methyl myristate, 10 mg of Methyl palmitate, 15 mg of Methyl stearate, 20 mg of Methyl arachidate, 20 mg of Methyl oleate, 10 mg of Methyl eicosenoate, 10 mg of Methyl behenate and 10 mg

of Methyl lignocerate into a 10 mL volutric flask. Added 5 mL of heptane and sonicated to dissolve. Diluted to volume with diluent and mixed well.

Reference solution B: Pipetted out 1.0 mL of Reference solution-A and diluted to 10 mL with diluent and mixed well.

Preparation of Reference solution-C:

Weighed and transferred 5 mg Methyl myristate, 10 mg of Methyl palmitate, 15 mg of Methyl stearate, 20 mg of Methyl arachidate, 20 mg of Methyl oleate, 10 mg of Methyl eicosenoate, 10 mg of Methyl behenate and 10 mg of Methyl lignocerate into a 10 mL volutric flask. Added 5 mL of heptane and sonicated to dissolve. Diluted to volume with diluent and mixed well.

Sample solution:

Weighed and transferred 0.1 g of polysaobate 80 into a 25mL bound bottom flak and added 2 mL of diluent. Boiled under reflux condenser at 100°C for 30 min. Added 2.0 mL of Boron trifluoride –methanol solution through the condenser and allowed to boil for 30 min. Added 4 mL of heptane through the condenser and boiled for 5 min. Allowed to cool to room temperature and added 10.0 mL of saturated sodium chloride solution and shaken for 15 sec. again added 10 mL of saturated sodium chloride solution. Collected 2 mL of the upper solution at the neck of the flask and washed with 2 mL of anhydrous sodium sulphate. Collected solution and used as sample solution and injected into GC.

Chromatographic conditions

GC parameters:

Column	: IC-Wax 0.32mm x 30 m, 0.5µm film thickness (Catalogue No.: 0711-3230-05)
Column ID	: SK-GC-007,
Brand Name	: Inert chrom
Oven temperature	: Initially maintain at 80.0 ° C and then rise to 220°C at the rate of 10.0°C /min, and hold for 40 minutes.
Column flow	: 4 ml / min*
Mode	: Constant Pressure
Detector Gas	: Air (350 ml/min)
Hydrogen	: (35 ml/min) *
Carrier Gas	: Helium
Make up Gas flow	: 30 ml/min
Linear velocity	: 50 cm/sec
Split ratio	: 1:50
Injection volume	: 1 µL
Injector Temperature: 250ºC	
Detector Temperature: 250ºC	
Equilibration time	: 1 µL
GC cycle time	: 54 min

System suitability:

Inject Blank (n-Heptane), Reference solution-A, Reference solution-B and reference solution-C and calculated the percentage of each component of the sample.

System suitability requirement:

Resolution should be not less than 5 for the peaks of methyl stearate and methyl oleate in reference solution-A

Theoretical plate count for the methyl stearate in reference solution –A should be not less than 30000.

Calculation:

= (Ac/At) x 100

Where,

Ac= Peak area of component of interest

At= Total area of all peaks related to fatty acids

Result:

S.No	Name of the Fatty acid	Limit (%)		Result (%)
1	Myristic acid	Not more than 5.0	-	3.4
2	Palmitic acid	Not more than 16.0	-	6.4
3	Palmitoleic acid	Not more than 8.0	-	4.2
4	Stearic acid	Not more than 6.0	-	2.3
5	Oleic acid	-	Not less than 58.0	75.9
6	Linoleic acid	Not more than 18.0	-	5.2
7	Linolenic acid	Not more than 4.0	-	1.0

Refer documentation: Page No. 34-43

14. Training of personnel:

Training had provided to concern personnel whoever involved in the execution of the analysis

15. References:

- Polysorbate 80 USP monograph
- USP general chapters

16. Certificate of analysis:

S. No.	Test	Specification	Result
1	Description	Lemon oily liquid	Complies
2	Solubility	Very soluble in water, soluble in alcohol and ethyl acetate and insoluble in mineral oil	Very soluble in water, soluble in alcohol and ethyl acetate and insoluble in mineral oil
3	Identification A	Complies with the test for Composition of Fatty Acids	Complies
	Identification B	The IR absorption spectrum of the preparation of the test specimen exhibits maxima only at the same wavelengths as that of a similar preparation of corresponding USP RS	The IR absorption spectrum of the preparation of the test specimen exhibits maxima at the same wavelengths as that of a similar preparation of corresponding USP RS
4	Residue on ignition (<281>) (%w/w)	0.25	0.19
5	Specific Gravity (<841>)	1.06 - 1.09	1.07
6	Viscosity (<911>) A. Temperature B. Kinematic viscosity	 24.9 - 25.1°C 300 -500 centistokes	 25 °C 432 centistokes
7	Fats and Fixed Oils-Hydroxyl value (<401>)	65 -80	69.4
8	Fats and Fixed Oils-Acid value (<401>)	Not more than 2.0	1.08
9	Fats and Fixed Oils-Peroxide value (<401>)	Not more than 10	7.77
10	Fats and Fixed Oils-Saponification value (<401>)	45 -55	48.9
11	Water determination (<921>)	Not more than 3.0%	2.2
12	Ethylene Oxide and Dioxane A. Ethylene oxide B. Dioxane	 Not more than 1 ppm Not more than 10ppm	 0.04 ppm Not detected
13	Assay-Composition of Fatty acids (%) A. Myristic acid B. Palmitic acid C. Palmitoleic acid D. Stearic acid E. Oleic acid F. Linoleic acid G. Linolenic acid	 A. Not more than 5.0 B. Not more than 16.0 C. Not more than 8.0 D. Not more than 6.0 E. Not less than 58.0 F. Not more than 18.0 G. Not more than 4	 A. 3.4 % B. 6.4 % C. 4.2 % D. 2.3 % E. 75.9 % F. 5.2 % G. 1.0 %

17. Handling and transit condition:

- Store in an Air tight container, protect from light

- Room temperature

18. Conclusion.

The material is tested as per the above test parameters in line with USP monograph and all the test parameter results are meeting with USP specifications.
Hence, the above test procedures are feasible to perform analysis in our laboratory.

19. Revision History

Version	Revision History	Effective date
00	New document	

END OF THE DOCUMENT