

# NK CHEMICALS PTE LTD

## QC STANDARD ANALYTICAL METHOD

TITLE : **FREE LANOLIN ALCOHOL** REVISION NO. : 1  
**CONTENT IN LANOLIN BY GAS** DATE OF ISSUE : 25/04/01  
**CHROMATOGRAPHY** PAGE : 1/4  
REF. NO. : SAM 43A

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### 1. DEFINITION

This is a clean-up of lanolin samples by GPC to remove Lanolin (esters) before analysis by FID Gas Chromatography for free lanolin alcohol content.

### 2. APPARATUS

#### FOR GPC :

- 2.1 Glass syringe, 10ml
- 2.2 Dropping pipette
- 2.3 Rotary flask, 200ml and 300ml
- 2.4 Volumetric flask, 10ml and 100ml
- 2.5 GPC column, Omnifit 25mm bore x 1250mm
- 2.6 GPC gel, Bio-beads SX-3, 200-400 mesh (40 - 80 um) from Bio-Rad Laboratories, 100g
- 2.7 Fraction Collector, Advantec model SF 2120
- 2.8 Sample injector, 6-port injection model V-541 valve, sample loop 5.00ml
- 2.9 GPC pump, FMI Lab from Fluid Metering Inc. Model QSY-1. Flow rate 4.00 – 4.10ml per minute
- 2.10 Disposable syringe filter, 0.45um pore size

#### FOR GC :

- 2.11 Gas Chromatography equipped with FID, Shimadzu GC-14A
- 2.12 Data Handler Shimadzu Chromatopac CR-8A
- 2.13 GC syringe, 10 microlitre

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2.14 GC column HP-1 or DB1, 0.32mm ID x 50m length x 0.17um film thickness

### 3. REAGENTS

3.1 Dichloromethane, residue analysis grade

3.2 n-Hexane, residue analysis grade

3.3 Iso-Octane, residue analysis grade

3.4 Methyl Ethyl Ketone (MEK), AR grade

3.5 USP Lanolin Alcohol RS, Lot F

### 4. PROCEDURE

4.1 Weigh 1.0000 to 1.0025g of lanolin sample into a 10ml volumetric flask.

4.2 Heat gently to melt the sample and dissolve with dichloromethane. Make up to mark.

4.3 Transfer the 10ml sample solution into a syringe with PTFE filter (0.45 micron) attached.

4.4 Inject about 9ml of sample into the GPC and make sure no bubble is introduced. Turn the injection knob to the "INJECT" position, simultaneously press the "START" button on the fraction collector.

#### 4.5 GPC TREATMENT - SETTING CONDITION

Elution Solvent : Dichloromethane  
Solvent flow rate : 4.00 - 4.10 ml per minute  
Sample Concentration: 1.000g per 10ml Dichromethane  
Injection Volume : 5.00 ml (fixed sample loop)

#### FRACTION COLLECTOR WINDOW SETTINGS :

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Wait Time 0 min 29 sec

<u>Window Number</u>		<u>Time Setting</u>	<u>Elution Fraction</u>
1	Window Starting Time	0 min 30 sec	Fraction 1
	Window Ending Time	58 min 30 sec	
2	Window Starting Time	58 min 31 sec	Fraction 2
	Window Ending Time	90 min 32 sec	

- 4.6 Collect fraction 1 & 2 in suitable rotary flask respectively.
- 4.7 Determine the oil content in fraction 1 by rotary evaporate off the dichloromethane. (if necessary)
- 4.8 Evaporate off the dichloromethane in fraction 2 using rotary evaporation at 40°C under N<sub>2</sub> purge to almost dryness.
- 4.9 Solvent exchange with 50ml hexane and continue evaporation to about 5ml.
- 4.10 Transfer the contents into a 100ml volumetric flask and rinse the rotary flask in hexane. Make up to 100ml with hexane.
- 4.11 The sample is then ready for free lanolin alcohol check using FID GC.

### 5. GC CONDITION

Column : HP-1 or DB1, 0.32mm x 50m x 0.17um  
Column Temperature Program : 220° - 280°C (3°C per minute)  
Injection Temperature : 270°C  
Detector Temperature : 300°C  
Carrier Gas : He, 6.8ml per minute (column)  
Make Up Gas : 80ml per minute (split)  
Hydrogen : 0.77 kg/cm<sup>2</sup>  
Air : 0.8 kg/cm<sup>2</sup>  
Stop Time : 45 minutes  
Split Ratio : 1 : 13

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Split Vent : 80.2 ml/min approx.  
Purge Vent : 22.7 ml/min approx.  
Standard Solution : USP Lanolin Alcohol RS Lot F  
0.0500 ± 0.0005g dissolve in 100ml hexane.

### 6. DETERMINATION OF FREE LANOLIN ALCOHOL - FID GC

- 6.1 Make sure the setting condition for GC-14A is already stabilised for analysis.
- 6.2 Inject 1ul of standard solution (USP Lanolin Alcohol RS Lot F) and then follow by 1ul of sample solution.

### 7. CALCULATION

$$\text{Free Lanolin Alcohol, \%} = \frac{100 \times C \times K \times r_u}{I \times W \times r_s}$$

where, C = concentration of USP Lanolin Alcohol RS in standard solution, *mg/ml*

I = volume injected into the gel permeation chromatography column, *ml*

W = weight of Lanolin sample taken, *g*

*r<sub>u</sub>* = Total Peak Area of Sample Solution

*r<sub>s</sub>* = Total Peak Area of Standard

K = corrected fraction of free lanolin alcohol in USP Lanolin Alcohol RS in the standard solution

where:  $K = 1 + (0.0062A - 0.0119S)$

A = acid value of USP Lanolin Alcohol RS

S = saponification value of USP Lanolin Alcohol RS

Reference : USP 2000 NF 19