	Method for Determination of Vitamin A in Edible Oil and Fats		
Method No.	**NA**	**Revision No. & Date**	NA
Scope	▪ Applicable for the Determination of Vitamin A in Edible Oil and Fats. The limit of Quantification is 0.1 mg/kg.		
Caution	▪ Vitamin A is sensitive to light, perform all steps under UV- shielded lighting. ▪ Dark room is required for sample Analysis and Standard Preparation ▪ Potassium Hydroxide is extremely caustic. This Chemical can cause severe burn Wear eye protection, gloves, and lab coat. Use only with adequate ventilation. Keep away from heat, sparks, and open flames.		
Principle	▪ Samples are saponified at high temperature, and then lipid-soluble components are extracted to petroleum ether. ▪ A portion of petroleum ether is transferred and washed with distilled water. ▪ The vitamin A adduct is subsequently reconstituted into a small volume of methanol and analyzed by reversed-phase liquid chromatography (RPLC) on C_{18} column, detected by ultraviolet (UV) detector at 325 nm		
Apparatus/Instruments	▪ High performance liquid chromatography: with Ultraviolet (UV) detector. ▪ Balance – Capable of accurately measuring weights from 0.05 to 100 g within ± 0.01 g. ▪ N_2 Concentrator- with nitrogen flow ▪ Water bath ▪ Vortex mixer/ rotary shaker ▪ Syringe with 0.2µm syringe filter ▪ Micro centrifuge vials. - 2 ml ▪ Reflux apparatus: Flat bottom flask (Amber color) 250mL ▪ Pipettes: graduated 100 - 1000 µl and 20 - 200µl ▪ Condenser ▪ Volumetric flask: 10mL and 100mL ▪ Beaker: 100mL ▪ Separating funnel:250mL ▪ Measuring Cylinder: 50 mL and 100mL		
Materials and Reagents/Standard	▪ Vitamin A (Retinol standards) ▪ Methanol: HPLC grade ▪ Water: Millipore Milli-Q system to>18 M-ohm resistivity, or equivalent. ▪ Petroleum Ether: AR Grade		

MoM – General
MoM - Pesticides
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(to be written depending upon concerned manual)

<table>
<tr><td></td><td>

KOH: AR Grade
Pyrogallol: AR Grade
Ethanol (95%)

</td></tr>
<tr><td>Preparation of Standards/Reagents</td><td>
<table>
<tr><th>S. No.</th><th>Compound</th><th>Make</th></tr>
<tr><td>1</td><td>Vitamin A</td><td>Sigma</td></tr>
</table>

Stock Solution: Transfer 10mg of Vitamin A Standard into 10mL Volumetric Flask and add 7mL Methanol and sonicate for 10 minutes. Maintain the volume as 10mL in Methanol and mix thoroughly. Label with name of Standard, Concentration, date of preparation, date of expiry. The stock standard solution is stable up to 15 days Standard.
Intermediate Standard Solution: Prepare the Intermediate Standard according to LOQ requirements and do the subsequent dilutions.

Working Standard Solution: Prepare the working standards from Intermediate standards according to the LOQ requirements and do the subsequent dilutions
Saponification solution – 50% potassium hydroxide (KOH). Dissolve 50g KOH in 100mL H2O
Antioxidant solution -1% pyrogallol. Dissolve 1.0 g pyrogallol in 100 mL ethanol.

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Sample Preparation and Method of analysis	▪ Take approximately 2±0.01 g of sample into 250 mL flat bottom flask (Amber Colour) ▪ Add 15 mL saponification solution (50% KOH). ▪ Add 20mL antioxidant solution (1% pyrogallol). ▪ Add 40mL ethanol, reflux for 45min at 90°C in Water bath. ▪ Remove sample and cool to room temperature ▪ Transfer sample into 250mL separatory funnels. ▪ Add 60 mL extraction solvent (Petroleum Ether) into funnel and shake well 5 min and separate upper layer into separate round bottom flask. ▪ Repeat the above step 3 times. ▪ Wash it with distilled water up to alkali free. ▪ Evaporate aliquot up to dryness under nitrogen gas. ▪ Dissolve the residue in 1mL methanol, as per requirement of the sample. ▪ Filter the solution with the 0.45µm (PVDF) syringe filter • Now inject 20µl of the filtered solution on HPLC system
Instruments Conditions	▪ LC Column: C18 (250mm×4.6mm) , 5µm ▪ Detector- Ultraviolet (UV) ▪ Wavelength-325 nm ▪ Mobile phase- A) Methanol (98%) B) Milli-Q (2%) Filter through a membrane (porosity 0.45 µm). ▪ Flow rate-1mL/min. ▪ Flow Type- Isocratic ▪ Column Temperature- Ambient ▪ Run Time- 10 min. ▪ Injection Volume- 20 µL
Calculation with units of expression	$Vitamin\ A\ (Retinol), ppm\ or \frac{mg}{Kg} = \frac{\text{Peak area of Unknown X Std. conc. (ppm) X Dilution (ml)}}{\text{Peak area of Std. area X Sample weight (gm)}}$ **Results with Recovery Correction**: Calculated concentration X Recovery factor
Inference (Qualitative Analysis)	Quantitative Analysis
References	▪ IS-15120:2002, Animal Feeding Stuffs Determination of Vitamin A ▪ Kienen et.al talanta 75 (2008) 141-146 ▪ AOAC 2001.13
Approved by	Scientific Panel on Methods of Sampling and Analysis

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<u>The following 'note' need to be added in all manuals:</u>

<u>Note: The test methods given in the manual are standardised/ validated/ taken from national or international methods or recognised specifications, however it would be the responsibility of the respective testing laboratory to verify the performance of these methods onsite and ensure that it gives proper results before putting these methods in to use".</u>

Editorials (For Reference purpose while writing methods)

Abbreviations to be used

Microgram		µg
Milligram		mg
Gram		g
Kilogram		kg
Milliliter		mL
Litre		L
Second	sec	
Minute	min	
Hour		h
Celsius	°C	
Kelvin		°K
Centimeter		cm
Millimeter		mm
Molar		M
Millimolar		mM
Micromolar		µM
Mole		mol
Normal	N	
Wavelength		nm

Some Editorials for the manuals

Space between numbers and units

- Mass and volume need spaces 12 g not 12g, 100 mL not 100mL

- Time also needs space 10 h not 10h, 15 min not 15min
- Temperatures need spaces
    - between value and degree sign: **37 ˚C**, not 37˚ C or 37˚C
    - but the degree sign for angles goes with the number: 90º angle
- Centrifugal forces need spaces
    - on both sides of the "×“ (remember not x)
    - 10,000 × g, not 10,000g or 10,000xg
- Other "places for spaces"
    - around equals sign: **n = 3**, not n=3
        - also around >, <, ~, etc
    - around plus/minus: 29 ± 7, not 29±7
- Percentages is the only exception
    - **5%** serum, **0.01%** bromophenol blue
    - This is because % is not really a unit, just an indication that the value is presented as the "ratio to 100"
    - **a <u>space</u> is required**: 10 mM or 6 M, never 10mM or 6M
- Use numerals to express numbers 10 and above.
- Use words to express numbers below 10.
- Use numerals when you have 3 or more numbers in a series, even if each of the numbers is below 10.
- When numbers begin a sentence, you must write them out in words.
- Situations in which Numbers Should be Given as Numerals

General Guideline	Examples
All numbers 10 and above	Trial 14; 35 animals; 16 genera of legumes
All numbers that immediately precede a unit of measurement	A wing 10 cm long; 5 mg of drug; 21days
Numbers with decimals; fractions that include whole numbers	7.38 mm; $4^1/_2$ hours
Numbers that represent statistical or mathematical functions or results, percentages, ratios	Multiply by 5; fewer than 6%; 3.75 times as many; the 2nd quartile
Numbers that represent exact times or dates; ages; size of samples, subsamples or populations; specific numbers of subjects in an experiment; scores and points on a scale; exact sums of money; and numerals as numerals	About 3 weeks ago, at 1:00 a.m. on January 25, 2000, the 25-year-old patients with IQ scores above 125 all awoke simultaneously in the nursing home at 125 Oak Street. They were paid $25 apiece to go back to sleep
Numbers below 10 that are grouped for comparison with numbers 10 and above in the same paragraph	4 of 16 analyses, the 1st and 15th of the 25 responses; lines 2 and 21
Numbers that denote a specific place in a numbered series, parts of books and tables, and each number in a list of four or more numbers	Trial 6; Grade 9 (but the ninth grade); the groups consisted of 5, 9, 1, and 4 animals respectively

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