AFDC 3(4793)P3 (REV.TZS 528:1992)

DRAFT TANZANIA STANDARD

Determination of Vitamin A (Retinol) in food and food stuffs- part 1- General routine method

TANZANIA BUREAU OF STANDARDS

0 Foreword

Vitamin A (Retinol)is one of nutritionally important growth factor required in the diet of human being and animals at a specific requirement level. Animals, fish, liver, oils, milk and butter are rich sources of vitamin A. Limited amounts are also found in vegetable oils and fats, meat and cereals.

The development of this standard was necessitated by the need to quantify vitamin A in foods and food stuffs.

Since this standard is for routine purpose, only high pressure liquid chromatographic (HPLC) Method shall be used for reference.

In preparation of this standard considerable assistance was derived from the *Agriculture Statutory Instruments*-11976 No. 840 published by the Ministry of Agriculture and Food, UK.

In reporting the results of a test or analysis made in accordance with this standard, if the final value observed or calculated, is to be rounded off it shall be done in accordance with TZS 4: (see clause 2).

1.0 Scope

This Tanzania standard specifies the general routine method for determination of vitamin A in food and food stuffs.The lower limit of determination is 3000 micrograms per kilogram for highly pigmented food and 1200 micrograms per kilogram for others. The products are classified in two groups, according to their pre-sumed retinol content.

Group A:Contents lower than 60000 micrograms per kilogram.

Group B: Contents equal to or greater than 6000 micrograms per kilogram.

2.0 References

For the purpose of this Tanzania standard the following references shall apply where by for dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

TZS 59: *Water for analytical laboratory use – Specification and test method*

TZS 4: *Rounding off numerical values.*

3.0 Principle

The sample is hydrolysed in hot ethanolic potasium hydroxide solution, either in the presence of an antioxidant or in a nitrogen atmosphere. The mixture is extracted with 1,2 -dichloroethane. The extract is evaporated to dryness and treated with light petroleum. The solution is chromatographed on acolumn of aluminium oxide. For group B products, chromatography is only required in certain cases. For group A products, the retinol is determined by development of coloured complex according to the Carr-Price reaction and measurement of its absorbance at 610-620 nm. For Group B products, the determination is by measurement of the absorbances at 325 nm.

4.0 Apparatus

4.1 Rotary vaccum evaporator

4.2 Glass chromatography column: preferably of Length 300 mm; internal diameter about 13 mm.

4.3 Spectrophotometer: With 10 mm cell, measurement in the UV require silica cells.

4.4 UV lamp: 365 nm.

5.0 Reagents

5.1 Quality reagents: Unless specified otherwise analytical grade chemicals and distilled water TZS 59 (see clause 2) shall be employed.

5.2 The following reagents are used for analysing products that belong to group A and B.

5.2.1 Aluminium oxide: Neutral (see 5.2.2). Ignite for 8 hours at 750^{o}C, cool in desicator and keep in a brown glass bottle fitted with a ground glass stopper. Before use in chromatography moisten as follows:

Place in brown glass bottle 10 g aluminium oxide and 0.9 ml water, seal with a stopper, reheat for 5 minutes in a boiling water bath while shaking. Allow to cool, verify the activity of aluminium oxide thus prepared by subjecting a known quantity of retinol (150 mg)to the procedure of (6.3) and (6.4) and checking recovery.

5.2.2 Aluminium oxide, basic. Degree of activity 1 with mesh size such that 5% is able to pass through a number 100 sieve; and absorption index of 30-40.

5.2.3 Dichloroethane.

5.2.4 Diethlyether. Remove peroxide and traces of water by chromatography on a column of basic of aluminium oxide (25g aluminium oxide per 250 ml diethlyether).

5.2.5 Ethanol 96-99% (v/v)

5.2.6 Nitrogen

5.2.7 ***Light petroleum***: Boiling range,40^{o} C-60^{o} C. If necessary purify as follows:

Stir 1000 ml light petroleum with 20 ml lots of concentrated sulphuric acid until the acid remains colourless.Remove the acid and wash the light petroleum successively with 500 ml water, twice with 250 m of sodium hydroxide solution (approximately 2.5 mol/l), and three times with 500 ml water.Remove the aqueus layer, dry the light petroleum for 1 hour over active carbon and anhydrous sodium sulphate, filter and distil.

5.2.8 Diethlyether solutions: Prepare a series of solutions containing 4, 8, 12, 16 and 20% (v/v)diethlyether in light petroleum.

5.2.9 Potassium hydroxide solution: Dissolve 500 g potassium hydroxide in water and dilute to 1 litre.

5.2.10 Sodium ascorbate solution: 10 g sodium ascorbate per 100 ml.

5.2.11 Sodium sulphide: 0.5 molar solution in 70% (v/v) glycerine.

5.2.12 Potassium hydroxide: 1 mol/l

5.2.13 Potassium hydroxide: 0.5 mol/l

5.3 The following reagents are used for analysing products that belong to group A.

5.3.1Toluene: Crystallizable

5.3.2 Chloroform**:** Remove the ethanol, phosgene and traces of water by chromatography on a column of basic aluminium oxide(50g aluminium oxide per 200 ml. Chloroform). It isadvisable to chromatograph the first 50 ml of elute a second time.

5.3.3 Carr- Price reagent: Stir approximately 25g antimony trichloride(kept in a desiccator) with a 100 ml chloroform until the solution is saturated. If necessary warm to 50^{o}C and allow to cool.A slight deposit of antimonytrichloride causes no problem. Add 2 ml acetic anhydride. Keep in refrigerator in a moisture-*proof brown glass bottle with ground glass stopper. The solution keeps for 2 to 3 weeks.*

5.3.4 Retinol; Standardized spectrophotometrically.

5.3.5 2-propan-ol: Used exclusively for analysing group B products.

6.0 Procedure

NOTE- All operations must be carried out away from direct sunlight using amber glass where necessary.

6.1 Test portion

From the prepared sample, take a quantity proportional to the presumed retinol content thus;10-1.0g for the content greater than 6,000,000 microgram/kilogram; 3.0-5.0g for content between 120,000 and 6,000,000 microgram/kilogram; 10-20g for contents between 60 000 and 120 000microgram/ kilogram; 30g for group A products. Immediately place the test sample in a 500 ml flask with a ground glass stopper.

6.2 Hydrolysis and extraction

For milk foods and products with a tendency to agglomerate or swell; double the quantity of the reagents shown in 6.2.1 and 6.2.2

6.2.1 Add successively to the sample 40 ml ethanol, 2ml sodium ascorbate solution, 10 ml potassium hydroxide solution (5.2.9) and 2 ml sodium sulphide solution. Sodium ascorbate need not be added when hydrolysis is carried out in nitrogen atmosphere.

6.2.2 Heat for 30 minutes at 70^{o}C - 80^{o}C under a reflux condenser and then cool under a stream of water. Add 50 ml ethanol and 100 ml 1,2-dichloroethane.Shake vigorously and then carefuly decant the supernatant liquid into the separating funnel avoiding transfer of solids. Add 150 ml potassium hydroxide solution(5.2.12), shake for 30 seconds and allow to stand until the layers have separated. Collect the lower dichloroethane layer in separating funnel. Add 40 ml potassium hydroxide solution, shake for 10 seconds and allow stand until the layer have separated. Collect the dichloethane layer in separating funnel, and wash at least 6 times with 40 ml lots of water. It is essential that the dichloroethane is free from alkali and washing must be continued untli the wash water gives no postive reaction to phenolphthalein. Collect the dichloroethane layer and remove the last traces of water using strips of filter paper.

6.2.3 Evaporate to dryness an aliquot part of the solution under vaccum on water bath at 40°C.Rapidly treat residue with 5 ml light petroleum. For group A products chromatograph as shown in 6.3.1. For group B products, transfer the solution to a 50 ml graduated flask, make up to volume with light petroleum, mix and measure the absorbances as shown in 6.4.1

6.3 Chromatography

***6.3.1 Group A products*:** Fill a chromatogaragh tube to a height of 200 m with aluminium oxide previously slurred with light petroleum. Place the tube solution obtained in 6.2 and immediately add 20 ml light petroleum. Elute successively with 10 ml lots of the light petroleum solution at 4, 8, 12, 16 and 20% diethlyether under pressure of partial vaccum, the rate of flow being to 2 to 3 drops per second.The carotene is eluted first. The retinol is generally eluted with light petroleum at 20% diethylether. The elution is followed under UV light (brief eradiation of the column with the mercury lamp). The fluorescent zone of the retinol is clearly separated from the yellow xanthophyll zones following it. If the zones have not separated, the chromatography should be repeated, using increased concentrations of diethylether in the eluting solvent. Collect the elute fraction containing the retinol in an Erlenmeyer flask.

***6.3.2 Group B products*:** Chromatography must be only carried out if the absorbance measurements obtained in 6.4.3 do not conform to the requirements given in 6.4.3. If Chromatography proves necessary, place in a Chromatography column an aliqout part of the solution in the light petroleum obtained 6.2, containing approximately 150 microgram retinol and Chromatography as shown in 6.3.1.

6.4 Determination

6.4.1 Group A products: Evaporate to dryness under vaccum the elute containing the retinol obtained in

6.3.1. Treat the residue with 2 ml benzene, using safety pipette. Take 0.3 ml of this solution and add 3ml of the Carr-Price reagent. A blue colur develops measure the maximum absorbance at 610 -620 nm exactly 30 seconds after the reaction has begun against a reference solution prepared from 0.3 ml of benzene and 3 ml of Carr-Price reagent. Determine the retinol content by reference to the standard curve (6.4.2).

***6.4.2 Calibration curve*:** Prepare in toluene a series of solutions of retinol containing 0.6 to 4.8 microgram per 0.3 ml. Treat this volume (0.3 ml) of each solution with 3 ml Carr- Priced reagent and measure that maximum absorbance at 610-620 nm.

***6.4.3 Group B products*:** Take an aliqout part of the solution in the light petroleum obtained in 6.2 containing approximately 60 microgram retinol. Evaporate to dryness under vaccum and dissolve the

residue in 25 ml 2-propan-ol. Measure the absorbance in the spectrophotometer at 325 and 334 nm. The maximum absorption is located at 325 nm. The retinol content of the solution is calculated as follows

Absorbance at 325nm x 5.49=microgram of retinol/ml.

however the ratio of the absorbances X/Y and Z/Y must be 0.857 where

X= absorbance at 310 nm

Y= absorbance at 325 nm and

Z= absorbance at 334 nm

If one of these ratios differ appreciably from this value(< 0.830 or > 0.880), the measurement of the absorbances must be preceded by chromatography in accordance with the method given in 6.3.2. If the measurement of the absorbances carried out after chromatography shows that the above mentioned ratios still differ appreciably from the value of 0.857(< 0.830 or > 0.880), the determination must be carried out in accordance with the method given for group A products.

7.0 Calculation of the results

Calculate of the retinol content of the sample taking into account the weight of the sample and the dilutions carried out in the course of the analysis. Express the results in microgram of retinol / per kilogram (kg) of the food sfuff.

8.0 Repeatability

The difference between the results of a determination in duplicate (obtained simultaneously or in rapid succession but the same analyst) shall not exceed 5%.