



East, Central and Southern African
Health Community

MANUAL FOR INTERNAL MONITORING OF OIL FORTIFIED WITH VITAMIN A

(Quality Assurance and Quality Control, QA/QC)

FIRST EDITION – 2007



**EAST, CENTRAL AND SOUTHERN HEALTH
COMMUNITY (ECSA-HC)**

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Foreword

Over the last five years, the East, Central and Southern African Health Community (ECSA-HC) has continued to undertake advocacy and technical assistance to assist member countries to embrace and scale up Food Fortification initiatives as a key strategy to reduce micronutrient malnutrition in the region.

ECSA has been working with partners in direct response to resolutions of the Conference of Health Ministers to scale up Food Fortification initiatives as a critical plank in fighting the devastating effects of micronutrient malnutrition among populations of member states. ECSA partners in the Regional Food Fortification Initiative include the A2Z Project, USAID, UNICEF, Micronutrient Initiative (MI), and ICCIDD, among others.

Part of the outcome of the intensified collaborative initiative, is a series of fortification guidelines developed to guide the Industry during the fortification process of staple foods and provide Government Food Inspectors a reference point in enforcing the standards.

Similarly, food control manuals have been developed for the Industry and the Government to provide technical reference resources that cover the entire fortification process to ensure that the fortified foods are safe and adequately fortified with the required fortificants.

This manual is part of a series of manuals on food fortification and is meant to directly contribute to the overall effort to strengthen food fortification in the region.

It is our hope that the use of this manual will help strengthen food control activities in our countries in order to deliver safe and quality fortified foods to the ECSA population.

Steven Shongwe
Executive Secretary
ECSA Health Community

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The manual is as a result of joint work by re-known food fortification experts. During the drafting of this manual, consultations with senior officers from food control departments of the ECSA member states were made and input incorporated.

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ECSA is deeply thankful to the above authors for preparing this manual.

Disclaimer

The content of this manual can be adapted to suit country specific contexts. In such a case, the content of the resulting document will be the sole responsibility of the organization adapting the manual and will not represent the views of the authors and that of the ECSA-HC. The Use of the content of this manual should be duly acknowledged.

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MANUAL FOR INTERNAL MONITORING OF OIL FORTIFIED WITH VITAMIN A (Quality Assurance and Quality Control, QA/QC)

Oil producers and importers play a key role in the oil fortification program because they are responsible for making sure that the product contains vitamin A in the specified amounts and properties. Quality control and assurance activities are vital to ensure that the fortified oil meets the requirements established in regulations and standards, from production to the market, as well as to avoid wasting resources. Quality assurance and quality control (QA/QC) for oil fortification does not require the implementation of a new program in factories, but only to incorporate into the ongoing QA/QC procedures those aspects that are specific to oil fortification. In any case, it requires the support and commitment of the general management to provide the human and financial resources to implement the new activities, and maintain acceptable levels of performance.

The fortification of oil with Vitamin A involves the blending of an oily fortificant normally containing high concentrations of retinyl palmitate (an active form of vitamin A). The yellow retinyl palmitate liquid is usually provided in 5 liter opaque containers to protect the retinyl palmitate from environmental factors. The fortificant is supplied in concentrated forms of 1.0 million IU/g or 1.7 million IU/g¹, which need to be diluted by a factor of approximately 10,000 to bring down the concentration to levels safe for consumption.

Most oil refineries were not designed with fortification in mind and so the position to incorporate the vitamin A should be defined on factory to factory basis. However, incorporation of the vitamin A is usually located after the deodorization process and before packaging. **Figure 1** shows a typical layout for oil fortification factory where one tank (smaller) may be used for pre-blending to prepare the premix (Tank A) and another for making the final dilution (Tank B), which in most cases is also the holding tank. Some factories add the premix directly to the Tank B, while others inject it into the refined oil when it is transported to the holding tanks. In any case, the dilution rate should correspond to the ratio between the content of vitamin A in the fortificant and the specified average content for the fortified oil during production.

The adequate mixing time must be determined for each blender and holding tanks by collecting a set of ten samples at intervals of 10, 20 and 30 minutes. The samples for each time interval are tested quantitative for vitamin A levels using a suitable analytical test, such as the UV spectrophotometer method that is presented in **Section E**. The mixing time is deemed adequate when the variation of vitamin A levels between samples for a specific time is less than 10%.

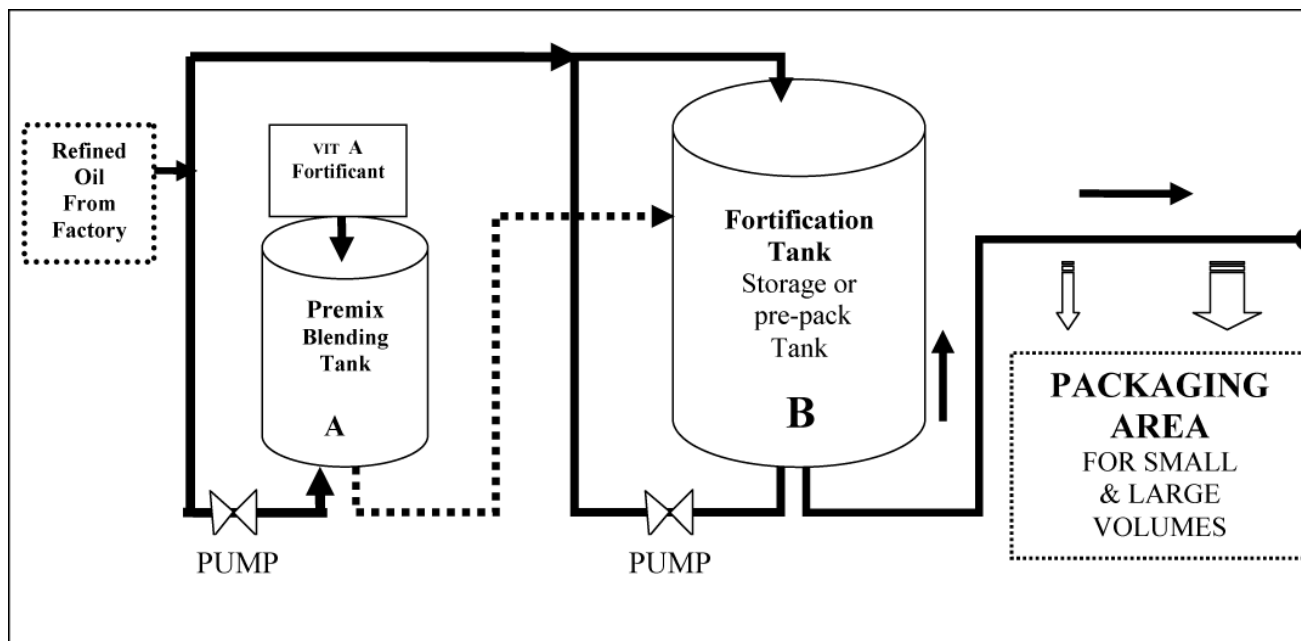


FIGURE 1: Blending and Holding Tanks for Fortification

This manual describes the steps to be carried out to ensure quality of oil fortified with vitamin A. In general, it covers the receipt, inspection and storage of the vitamin A fortificant; the two-step dilution process of vitamin A into oil; and the confirmation of the technical specifications. The manual also includes sections that describe analytical methods to determine vitamin A in oil.

¹ Equivalent to 300 grams of retinol per kilogram, and 510 grams of retinol per kilogram, respectively.

As any other QA/QC system, identifying the causes of non-compliance, implementing corrective and preventive actions is indispensable, as well as keeping updated records of the activities performed. National health authorities visit oil factories to carry out technical audits and inspection to the fortification process and product. These government activities are mainly centered on checking the producer's records. Therefore, it is important to keep in mind that whatever is done is recorded appropriately. The following sections are included in this manual:

- Quality assurance of the vitamin A compound procurement, receipt, storage and delivery
- Quality assurance of the oil fortification process
- Quality control of the fortified oil
- Semi-quantitative method to determine vitamin A in fortified oil
- Quantitative spectrophotometric method for determining vitamin A in fortified oil

A. QUALITY ASSURANCE OF THE VITAMIN A COMPOUND PROCUREMENT, RECEIPT, STORAGE AND DELIVERY

I. Objectives and Accountability

The purpose of the Quality Assurance of the vitamin A receipt, storage and delivery are to ensure that:

- The factory always has enough vitamin A compound in stock for at least 3 months of production of fortified oil.
- Vitamin A compound is stored under adequate conditions and is used based on the “first-in, first-out” (FIFO), as determined by the expiration date.

Those directly responsible for achieving these objectives are the *Warehouse Manager* and the *Head of the Quality Control Department*, who should frequently inform the *Factory Manager*.

II. Procedures

a. Procurement of Vitamin A Compound (Fortificant)

Fortificant should be procured from nationally approved sources and adequate stocks should be available at all times based on production.

b. Receipt and Storage (warehouse)

1. Every time a new lot of vitamin A compound is received in the factory, check that the containers are hermetically sealed and that a Certificate of Analysis (COA) has been included.
2. Record in a form similar to **Table A-1** the number of containers received, lot numbers, expiry date, and the name of the person who is receiving the delivery.
3. Store the containers in a clean, cool dry area and away from chemical products or other potential contaminants. If possible, store the vitamin A containers in an air conditioned room.
4. Stack the containers in such a way that the first to expire¹ are used first, following the “first-in, first-out” system.

c. Delivery (warehouse)

1. When a vitamin A container is dispatched for oil fortification, record the date of dispatch and name of the person who is receiving the order, as shown in **Table A-1**.
2. Send a copy of the log form every week to the Quality Control Department and the Production Manager.

d. Confirming proper use and vitamin A content of the fortificant (Quality Control Department)

1. At least once a week, an employee of the Quality Control Department visits the warehouse and the fortification area to ensure that vitamin A is being used in the order of expiration date, and that records are kept up to date. Reviewer must sign in last column of **Table A-1**.
2. At least once a month, take two 30 g samples from the cans that will be used the day of sampling. Package them in opaque airtight container and send them to an external laboratory to confirm the vitamin A content.
3. When result is available report it to the Production Manager.
4. If the results are below the claimed content, contact the vitamin A supplier.

III. Records and Reporting

Warehouse responsible should keep updated all the records, which should be periodically reviewed by personnel from the *Quality Control Department*. Weekly reports should be sent to the *Factory Manager* and the Quality Control department, where the reports will be filed, too.

¹ Precaution should be taken to avoid having product in storage with expiration dates shorter than 6 months.

B. QUALITY ASSURANCE OF THE OIL FORTIFICATION PROCESS

I. Objectives and Accountability

The purpose of Quality Assurance of the oil fortification process is to ensure that adequate quantities of vitamin A are added to the oil to meet the requirements of the national standard. The following activities are therefore important

1. Equipment is adequately calibrated and the pumping system delivers the vitamin A premix without leakages or delays.
2. Ratio oil produced (MT)/vitamin A compound (kg) is close to the theoretical ratio based on quantities used.
3. Vitamin A compound is adequately diluted taking into account the most recent laboratory report of the vitamin A content in the fortificant (vitamin A compound).

The responsible people for this component are the *production personnel* assigned to the area where fortification and packaging are taking place, lead by the *Production Manager*. *Quality Control Department* is in charge of supervising the activities and daily or weekly reporting to the *Factory Manager*.

II. Procedures

Typically, the vitamin A fortificant contains higher levels of vitamin A than declared in the label. This additional vitamin A is referred to as an *overage* and it is meant to cater for any losses than may occur during transportation and storage. The Certificate of Analysis shows the actual level of vitamin A and this should be used to calculate dilution levels. It is also necessary to confirm frequently the vitamin A content of the final product in an external laboratory (see **section C**).

a. Calculating the amount of vitamin A compound per batch (Production Manager) **Batch System**

1. Once the result of the content of vitamin A of the fortificant is received from the laboratory, estimate the amount of the fortificant that

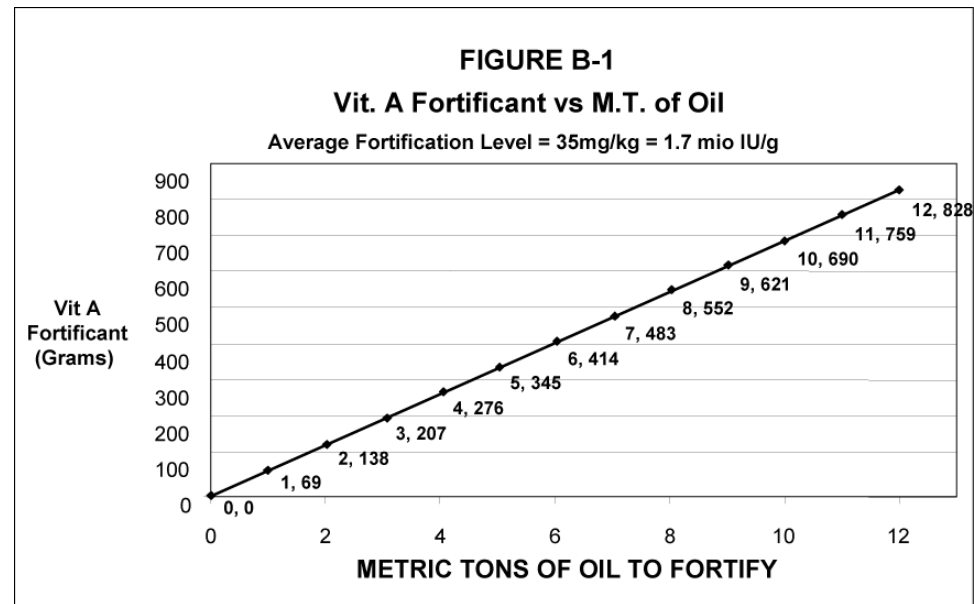
is necessary to fortify one metric ton (1,000 kg) of oil, using the equation below:

$$\text{Fortificant (g/MT oil)} = \left[\frac{\text{Average Vit. content in oil (mg/kg)}}{\text{Vit. A content in fortificant (g/kg)}^1} \right] \times 1000$$

For example, if the required average content of vitamin A is 35 mg/kg (3.5 mg/100 g or 116 IU/g), and the fortificant has a vitamin A content of 1.7 million IU/g (510 g/kg), the amount of fortificant to use per metric ton of oil is:

$$\text{Fortificant (g/MT oil)} = [35 \text{ (mg/kg)} / 510 \text{ (g/kg)}] \times 1000 = 69 \text{ g/MT}$$

1. Multiply the expected production in metric ton by the calculated amount of vitamin A per metric ton. A chart, such as that illustrated in **Fig. B-1** will help to confirm the calculation. The chart below is based on an average vitamin A level of 35 mg/kg, while the Vitamin A fortificant has a concentration of 1.7 million IU/g (510 g retinol/kg).



- a. Making the premix (first dilution)** (Production Personnel)
 1. Weigh accurately the appropriate amount of the fortificant (vitamin A compound) as estimated above. Use stainless steel equipment and handle the weighing process following good manufacturing practices for food safety.

2. Add the fortificant into the blending tank, and mix for 10, 20 or 30 minutes depending on the adequate mixing time determined at the factory as described in the introduction. Record amount of fortificant that was used, as well as the time when mixing started and when mixing ended in **Table B-1**.
3. Discharge premix into the holding tank. Record the time in **Table B-1**. Data should always be ready to show to the Quality Control Department when requested. When a form is completely filled-out, send a copy to the Quality Control department.
4. Weekly, check the performance of the balance, pump, and the integrity of the feeding tubes and the blending tank. Record the results of this activity in **Table B-2**.

I. Records and Reporting

The Production department should keep updated and adequately filed records of the calculations done, amounts of oil produced and amounts of fortificant used, as well as description of actions taken during production to keep the fortification process performing as expected. A copy of these records will be sent daily to the Quality Control Department.

C. QUALITY CONTROL OF FORTIFIED OIL

I. Objectives and Accountability

The purpose of Quality Control of the fortified oil is to ensure that:

- All oil samples contain the regulatory minimum level (i.e. **> 10 mg/kg**)¹.
- 80% of samples contain vitamin A within regulatory levels of **10 to 45mg/kg** vitamin A and the average is close to the factory addition level of 30mg/kg.
- Fortified oil is packaged and labeled as required in the National Standards for General Labeling of Prepackaged Foods and the Oil Fortification Regulations.

The responsibility for this component is the *Quality Control Department*, which should send daily reports to the *Production Manager*.

II. Procedures

a. Collecting samples for quality control (Packaging Department)

1. Prepare a composite sample by collecting 200 mL of oil every hour, and placing in an opaque 2-L container.
2. At the end of the day label the sample with the date, hour and number of batch or batches. Include the amount of oil (in kilograms) produced during the period. Send sample to the Quality Control Department.

b. Vitamin A determination

Semi-quantitative analysis should be done hourly for each sample. In the laboratory, mix well and take 50g to use for the determination of retinol concentration using the “Semi-quantitative method for determining retinol in fortified oil”, or the “Spectrophotometric quantitative method” if the necessary equipment is available (see **Sections D** and **E** for the Analytical Methods).

Based on ECSA 2007 guidelines for oil fortification.

1. Record results in the chart of **Table C-1**, expressing them in terms of vitamin A (retinol). If the semi-quantitative method is used, apply the ranges: 0-10 mg/kg, 10-20 mg/kg, 20-30 mg/kg, 30-40 mg/kg and >40 mg/kg.

2. Prepare a **daily** composite sample, mixing 100 mL from each of the hourly samples. Mix well. Determine the content of vitamin A for the daily composite sample and record result in **Table C-1**. Store the remaining daily-composite sample in an air-tight and opaque container. Identify the sample with the date, and include the amount of vitamin A from semi-quantitative testing of the *Daily Comp.* sample. Keep this sample in the sample-store room for up to a month.

a. Supervision (Department of Quality Control)

1. Make unannounced visits to the fortification place to check that the operators are following instructions and the records are being filled out timely. Sign **Table B-1** to confirm completion of this supervision.
2. Make unannounced visit to the packaging site to verify that the operators are taking 200-mL of oil every hour, and mixing the samples to prepare a daily composite sample. The sample should be labeled with the date and time of preparation.

b. Corrective actions

If abnormalities are found, discuss immediately with the Production Manager the corrective actions to be taken.

I. Records and Reporting

7. Complete **Table C-1** with the data provided by the production (Table B-1) department.
8. Calculate the ratio oil produced/vitamin A compound. The ratio should be close to 9 to 15, when expressing oil production in kilograms and amount of fortificant used in grams, depending on the type of vitamin A compound that is being used.
9. Record all the needed information in **Table C-1**, and send a daily a copy to the production manager for attention and filing.
10. At least once a month, select randomly two daily-composite samples from the sample store and send to an external reference laboratory for the quantitative determination of vitamin A. Send also unfortified oil, which will be used as the blank for the laboratory.
11. Once results are received, record those in the corresponding **Table C-1**. Compare the results with your own data, and if incompatibility is found look for the reason, and apply corrective measures as needed.
12. Send reports to the production manager about corrective actions or confirmation of the earlier findings and deductions from the work of the Quality Control Department.

D. SEMI-QUANTITATIVE METHOD FOR DETERMINING VITAMIN A IN FORTIFIED OIL

I. References

Bayfield, R.F. and E.R. Cole. (1980). Colorimetric Determination of vitamin A with Trichloroacetic Acid. In: *Methods in Enzymology*, vol. 67. Vitamins and Coenzymes, Part F. Eds: McCormick, D.B. and L.D. Wright. Academic Press, New York. pp 189-195.

Kakuda Y, Firouz J, Varghese A. Comparison of colorimetric assays for vitamin A determination in fortified oil, sugar, salt, flour to HPLC method. No published, 2003.

II. Principle

The method described is based on the formation of anhydroretinol when retinol or its esters reacts with a chromogenic solution made by dissolving trifluoroacetic acid (TFA) in dichloromethane (DCM). A blue complex is formed and the intensity of the color is proportional to the amount of retinol which can be measured semi-quantitatively by visual comparison against a reference scale of standard copper sulfate solutions. The blue color is transient, so the comparison should be done within 10 seconds of adding the reagent. Other compounds can replace TFA, such as trichloroacetic acid (TCA) and antimony trichloride (Carr Price solution) (see section F). However, TFA has proved to be easier to handle and does not run cloudy due to moisture absorption as does TCA under humid conditions. DCM is preferred but other solvents such as hexane or chloroform may also be used.

III. Critical Points and Cautions

The chromogenic reagent has to be prepared frequently because it is unstable. The reagent should be used within 5 days if stored at room temperature and within 14 days if refrigerated. If acetic anhydride is added to the solution, the chromogenic reagent is stable at room temperature for at least 18 days. If refrigerated, it should be removed from the refrigerator 2 to 3 hours prior to use. If necessary, it can be warmed in a water bath between 30-40°C. If crystals develop, they can be dissolved by manual agitation of the container. To verify the quality of the reagent, a control with a known concentration of vitamin A in oil should be analyzed at the same time, and the intensity of the blue color should match the expected intensity according to the reference scale.

The chromogenic reagent is corrosive and should be handled with care by trained personnel and protective clothing and gloves must be worn. Immediately before use, the volume required should be transferred to a beaker, from where it can be drawn into a syringe before being added to oil. A syringe rather than a pipette is used because the addition of the reagent should be vigorous and rapid. In addition, the beaker into which it is poured must be dry and at room temperature. Any reagent in the beaker that is not used should be discarded appropriately and NOT returned to its original container.

IV. Equipment and Materials

- Balance
- Colorimetric scale of standard copper sulfate solutions
- Disposable rubber gloves
- Glass test tubes (15mm x 100mm)
- Pasteur pipettes and pipettes bulbs
- Wide mouth glass bottle (to collect used reagent)
- Beaker (50-100mL)
- Dark glass bottle with glass stopper
- Glass syringe (5-10mL) with 3 cm Teflon¹ tip
- Graduated pipettes (5-10 mL)
- Volumetric flask, amber (50 mL)

V. Reagents

a. Chromogenic reagent: Trifluoroacetic acid/Dichloromethane

Mix 30.0 mL trifluoroacetic acid (FW: 114.03, 99.5%) in 60 mL dichloromethane (FW: 84.93, 99.5%, d=1.32 g/mL; DCM is also referred to as methylene dichloride). Store in a brown bottle in a cool environment. When properly stored, the solution has been found to be stable for up to 4 months. The chromogenic reagent prepared as stated is sufficient for 25-30 samples.

¹ The tip material must be resistant to dichloromethane

- Colorimetric scale

Prepare the following dilutions from a 300g/L stock solution of copper sulfate ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$).

Volume (mL) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -300 g/L to prepare 10 mL	Concentration of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ (g/L)	Approximate Concentration of Retinol (mg/kg)
1	30	10
2	60	20
3	90	30
4	120	40

Make up to volume (10 mL) with distilled water.

Measure 5mL of each of the copper sulfate standard solutions into clearly labeled tubes and the type of tubes should be the same as those in which the samples will be analyzed. Close the tubes tightly using a rubber stopper or a screw cap. It is better if the tubes are completely sealed to avoid evaporation of the solution. The color intensity of these copper sulfate solutions are calibrated against the color produced by the reaction of standards retinyl acetate solutions with the chromogenic reagents. It is preferable to express the results in terms of retinol instead of retinyl esters and hence the scale is presented in terms of milligrams of retinol per kilogram.

Identify each tube with a number indicating the **concentration of retinol in mg/kg** that the color represents. These standard solutions are stable and can be kept indefinitely at room temperature.

Other chromogenic reagents may be used such as trichloroacetic acid (TCA) and antimony trichloride (Carr Price Solution) as described below.

a. **Trichloroacetic acid (TCA)**: Dissolve 25 g of TCA (FW: 163.39) in 35 mL of dichloromethane and heat gently to dissolve. Make up the solution to 50 mL with the solvent. Acetic anhydride (15 mL) is also added to increase the stability of the solution normally affected by the presence of moisture. Store in a brown bottle. TCA is readily available and a low cost reagent, but it is corrosive and the complexes formed are less stable than TFA complexes.

b. **Antimony trichloride (Carr Price solution)**: The solution is prepared by dissolving 100 g of antimony trichloride (SbCl_3 , molar mass 228.11) in 300 mL chloroform. Acetic anhydride (15 mL) is also added to increase the stability of the solution normally affected by the presence of moisture. Care should be taken to keep the reagent as dry as possible and away from light.

I. Procedure

a. Diluting the oil sample

1. Place a 50 mL amber volumetric flask on a balance. Amber flasks should be used because vitamin A is light sensitive.
2. Tare the flask and transfer 10.0 g of oil into the flask using a Pasteur pipette. Accurately record the mass to one decimal place.
3. Add DCM to the flask to dissolve the oil and make up to volume and mix thoroughly.

b. Reaction with the chromogenic solution

4. In a tube of similar dimensions to those used for the copper sulfate solutions, pipette 3.0 mL of the TFA solution, and stand next to the tubes containing the copper sulfate solutions.
5. Into this tube containing the TFA, inject (a syringe can be used) rapidly 1.0 mL of the diluted sample solution of oil and mix quickly on a vortex.

c. Interpreting the result

6. Compare the color intensity developed against the set of tubes of copper sulfate within 5 to 10 seconds.
7. Estimate the approximate concentration of retinol in the oil sample by matching the color developed to the closest tube in the reference scale. In most instances, the intensity of the blue color of the sample will fall between two of the reference tubes. The level of retinol in oil should be reported as falling within the range corresponding to the reference tubes. For example, if the intensity of the blue sample solution lies somewhere between the levels of 30 and 60 g/L copper sulfate standard solutions, the retinol level is between 10 and 20 mg/kg. Do not attempt to be more precise.

d. Discharging the used reagents

8. Discard residual chromogenic reagent, including the oil-reagent mixture, into a glass bottle containing dissolved sodium bicarbonate, slowly adding the reagent to the bottle. The bottle should be clearly labeled as a waste bottle.
9. After the bottle is filled, the content can be discarded appropriately in line with local regulations for disposal of hazardous waste. It is recommended to burn it in a chemical incinerator equipped with an after burner and scrubber.

E. QUANTITATIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINING VITAMIN A IN FORTIFIED OIL

I. References

Kambagar, T & Fawzi, A.B. (1978). Spectrophotometric Determination of Vitamin A in Oils and Fats. *J Assoc of Anal Chem.* 61(3):753-755.

Subramanyam, G.B. & Parrish, D.B. (1976) Colorimetric Reagents for Determining Vitamin A in Feeds and Foods. *J Assoc Off Anal Chem.* 59(5):1125-1136.

II. Principle

The method is applicable to oils fortified with vitamin A in the form of retinyl palmitate or retinyl acetate, and it is based on absorbance of retinol within the UV-VIS region. Retinol and its esters absorb UV radiation with a maximum of 325 nm. Retinyl esters in the fortified oil are determined by diluting the oil in organic solvents such as dichloromethane, chloroform or hexane, followed by reading the absorbance of the solution at 325 nm. The concentration of retinol is estimated by dividing the absorbance with the extinction coefficient of retinol and its esters in the different solvents. Other substances naturally present in oil such as carotenoids absorb close to 325 nm and so absorbance must be corrected for a blank absorbance of the specific oil using unfortified oil from the same batch. Another option is to read the absorbance of the sample solution before and after exposure to ultraviolet irradiation. The difference between the two readings is associated with retinyl ester which is destroyed by the UV-irradiation.

III. Critical Points and Cautions

A spectrophotometer capable of accurately reading absorbance at 325 nm is essential. Given the importance of the spectrophotometer for ensuring the accuracy and reliability of the retinol determinations, it should be calibrated frequently following the instructions provided by the manufacturer, especially to confirm the calibration of the monochromator. This confirmation should be carried out frequently and not only when a new lamp is installed. Low actinic(amber) glassware should be used in the analysis, but if not available, protect samples and glassware containing the samples solutions from light with a piece of black clothing, aluminium foil or install gold-fluorescent light (yellow light) in the room.

IV. Equipment and Materials

- UV Spectrophotometer (325 nm)
- Vortex mixer
- Beaker (250 mL)
- Black clothing
- Pasteur pipettes
- Spectrophotometer quartz cuvettes (UV)
- Volumetric flasks, amber (50 mL)
- Graduate pipettes (to measure 2, 3 and 8 mL)

V. Reagents

- Dichloromethane (FW= 84.93, 99.5%, d=1.32 g/mL) or
- Hexane AR. (C₆H₁₄), purity=99%, FW=86.18, d=0.66 g/mL.

VI. Procedure

a. Diluting the oil sample

1. Place a 50 mL volumetric flask on a balance. Amber flasks should be used because vitamin A is light sensitive.
2. Tare the flask and transfer 1.0 g of oil into the flask using a Pasteur pipette. Accurately record the mass to four decimal places.
3. Add solvent (preferred dichloromethane) to the flask to dissolve the oil and make up to volume and mix thoroughly.
4. Repeat the process above using blank oil (unfortified oil from same batch).

b. Reading the absorbance of samples and unfortified controls

5. Place the solvent used for diluting the samples into 1 cm quartz UV cuvettes and zero the spectrophotometer at 325 nm. Use the solvent as the spectrophotometric blank.
6. Record the absorbance of samples and unfortified controls at 325 nm.

VII. Calculations

1. Correct reading of the samples by subtracting the absorbance of the unfortified oil treated in a similar manner. This is the corrected absorbance for the sample to be used for calculations.
2. Estimate the retinyl palmitate concentration of the oil sample using the following equation:

$$retinyl\ palmitate(mg/kg) = \frac{Abs_{corrected} \times Vf \times CF_{spec}}{a \times w}$$

Where $Abs_{corrected} = Abs_{sample} - Abs_{unfortified\ oil}$ and $Abs_{unfortified\ oil}$ is the average absorbance of the unfortified oil treated in similar manner as the samples.

The equation parameters are:

PARAMETER	EXPLANATION	VALUE
a	Retinyl palmitate absorption coefficient in dichloromethane ($mg^{-1} cm^{-1} L$).....	0.094
	or in hexane ($mg^{-1} cm^{-1} L$).....	0.092
Vf	Final volume (mL)	50
w	Weight of the sample (g).....	data from weight
CF _{spec}	Correction factor of the spectrophotometer. Ideally.....	1

To express the results as unesterified retinol, the ratio of the molecular weights of retinol/retinyl palmitate ($286.46/524.84 = 0.546$), must be taken into consideration. A simplified equation to estimate the unesterified retinol, when hexane is used as solvent, is:

$$retinol\ (mg/kg) = Abs_{corrected} \times \frac{290.4}{w} \times CF_{spec}$$

I. Alternative procedure: Irradiation with UV light in the irradiation chamber¹

1. Place about 2.5 mL of the diluted samples into a 10 mm x 75 mm glass test tube transparent to UV light and close it with a cap resistant to dichloromethane or hexane.
2. Irradiate the tubes in the irradiation chamber for 35 minutes (or the time required according to the performance of the irradiation chamber, see **Annex 4**).
3. Adjust the zero of the spectrophotometer with the solvent. Read the absorbance of the irradiated and unirradiated solutions at 325 nm in 1 cm light path quartz cuvettes.
4. Calculate vitamin A concentration with the following equation:

$$\text{where Abs}_{\text{corrected}} \text{ is: } \text{Abs}_{\text{corrected}} = (\text{Abs unirradiated sample}) - (\text{Abs irradiated sample})$$

¹ A model of this irradiation chamber is presented in **Annex 3**.

OIL FORTIFICATION: TABLE B-2

WEEKLY CHECK UP OF EQUIPMENT USED IN OIL FORTIFICATION WITH VITAMIN A

Date: _____

Page No. _____

EQUIPMENT/DEVICE	CONDITION ¹ (√)/(X)	OBSERVATIONS
1. Blender		
2. Balance		
3. Pump		
4. Stirrers		
5. Feeding tubes		
6. Blending tank		

Name/Signature: _____

¹ Condition: (√) = adequate, (X) = inadequate



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