East, Central and Southern African
Health Community

MANUAL FOR INTERNAL MONITORING OF SUGAR 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 strategy 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 theindustry 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

Acknowledgement

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The manual is as a result of joint work by distinguished food fortification experts in developing countries. 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.

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

Sugar producers, packers and importers play a key role in the sugar fortification program, since 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 sugar meets the requirements established in standards and regulations, from production to the market, as well as to avoid wasting resources. Quality assurance and quality control (QA/QC) for sugar fortification does not require the implementation of a new program in factories or packaging centers, but only to incorporate into the ongoing QA/QC procedures those aspects that are specific to sugar 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.

This manual describes the steps to be carried out to ensure quality of sugar fortified with vitamin A in any sugar factory or packaging center that fortifies sugar. In general, it covers the receipt, inspection and timely delivery of the vitamin A premix; the sugar fortification process; and quality control of the fortified sugar. The manual also includes a section that describes analytical methods to determine vitamin A in sugar. The fulfillment of these activities demands the coordinated participation of personnel from the Warehouse, Production, and Quality Control and Quality Assurance departments.

Each stage of the process and quality control of the product includes listing of objectives measured by indicators and criteria of success, and responsible persons. 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 sugar factories to carry out technical audits and inspection to the fortification process and product. Those government activities are mainly based on checking the producer's records. Therefore, it is important to keep in mind that "what has not been recorded has not been done".

The following sections are included in this manual:

- Quality assurance of premix receipt, storage and delivery
- Quality assurance of sugar fortification process
- Quality control of the fortified sugar
- Semi-quantitative method to determine vitamin A in fortified sugar
- Quantitative spectrophotometric method to determine vitamin A in fortified sugar

A. QUALITY ASSURANCE OF PREMIX RECEIPT, STORAGE AND DELIVERY

I. Objectives and Accountability

The purpose of the Quality Assurance of premix receipt, storage and delivery is to ensure that:

- The factory always has enough vitamin A premix inventory for at least 15 days of production of fortified sugar.
- Premix is stored under adequate conditions and is used based on the “first-in, first-out”.
- Premix bags are sealed and labeled, and they contain the minimum retinol level that is claimed.

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* and this, could even be on a daily basis if the production is large.

II. Procedures

a. Receipt and Storage (warehouse)

1. Every time a new lot of vitamin A premix is received in the factory, check that the bags are properly sealed and the label contains the following: manufacturing company, the lot number, date of production, and the vitamin A content.

2. Record in a form similar to **Table A-1** the number of bags received, lot numbers, date of production, date of receipt, and the name of the person who is receiving the delivery.

3. Store the premix bags on top of palettes made of a suitable material[1], in a clean dry area and away from chemical products or other potential contaminants. If possible, store the premix bags in an air conditioned room.

[1] Palettes should be made of a suitable material to maintain safety of food. If wooden palettes are used, handling and proper storage of them must be assured in order to prevent pest infestation and contamination of food.

4. Stack the bags in such a way that the storage and dispatch follows the “first-in, first-out” system.

b. Delivery (warehouse**)**

5. When premix is dispatched for sugar fortification, record the date of dispatch and name of the person who is receiving the order, as shown in **Table A-1**.

6. Send a copy of the log form every week to the Quality Control Department and the Production Manager.

c. Confirming content of vitamin A in the premix (Quality Control Department**)**

7. Once in a while, an employee of the Quality Control Department visits the warehouse and the fortification site to ensure that premix is being used in the order of delivery, and that records are being kept up to date. Supervisor must sign in last column of **Table A-1**.

8. At least once a month, take two 50 g samples from a lot of premix received**.** Package them in an opaque airtight container and send them to an external laboratory to confirm the vitamin A content.

9. Record the analytical results in **Table A-1** completed by the warehouse department.

10. If the results do not meet the premix specifications, contact the vitamin A premix 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.

B. QUALITY ASSURANCE OF THE SUGAR FORTIFICATION PROCESS

I. Objectives and Accountability

The purpose of Quality Assurance of the sugar fortification process is to ensure that:

- Premix is free flowing and adequately added to the unfortified sugar, either manually or using a feeder equipment.
- Feeder is dispensing premix adequately as verified by the amount of premix discharged in relation to the sugar flow.
- Ratio sugar produced (MT)/premix used (kg) is close to 2.0 (or 1,000 if ratio is expressed in terms of 50 kg-bags of fortified sugar per 25-kg bags of premix)[1].

The responsible persons for this component are the *production personnel* assigned to the area where fortification is taking place, with supervision by the *Quality Control Department*, and daily or weekly reporting to the *Factory Manager.*

II. Procedures

a. Beginning of the shift

1. Check that only the approximate amount of premix to be used per shift is in the fortification site. Open the bags the moment they are to be used.
2. *Feeder verification*[2]: Collect the amount of premix discharged by the feeder for one minute. Repeat this step three times.

[1] This proportion is valid for sugar fortified at 7.5-8.0 mg/kg. If fortification level is 15 mg/kg, then the ratio should be 1.0 (or 500 if expressed in terms of 50 kg-bags of fortified sugar per 25-kg bags of premix).

[2] Check the feeder at least once every shift or frequently as needed during the day.

3. Weigh the three portions collected and calculate the mean, standard deviation and coefficient of variation[3]. If coefficient of variation is higher than 5%, collect a fourth portion of premix for one minute and calculate the mean again.

4. Compare the amount of premix discharged by the feeder expressed in (g/min) to the theoretical amount that should be added according to the current sugar production rate in the factory.

5. If the amount discharged does not coincide with the theoretical one, adjust the feeder and repeat steps 2 to 4 again. Record results in **Table B-1**. Keep it ready to show to the Quality Control Department when required.

b. During the shift

6. Check periodically that the feeder is loaded with enough premix, and that it is working properly.

7. Take 500 grams of sugar every hour (or every 30 minutes if production is high), mix well and detect the presence of vitamin A using a qualitative assay (see **Section D**).

8. Report any abnormality to supervisor immediately.

c. End of the shift

8. When the shift (8-hours) ends, or more frequent if production is high, mix well eight single samples to prepare a composite sample, and label it with the date, hour and number of batch or batches. Send samples to the laboratory.

9. Record in **Table B-2** the amounts of sugar produced and the quantities of premix used (including identification of the bags) during the shift. Records can be done more than once during the same shift

[3] CV (%) = Mean/Standard Deviation x 100

III. Records and Reporting

Supervisor of the fortification process should keep updated and adequately filed records of the feeder verification, amounts of sugar produced and amounts of premix used, as well as description of actions taken during production to keep the fortification process performing as expected.

C. QUALITY CONTROL OF FORTIFIED SUGAR

I. Objectives and Accountability

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

- All sugar samples contain the minimum regulatory level of vitamin A (e.g. **>2mg/kg**[4]).
- 80% of all samples to contain vitamin A levels for factory specifications (e.g. **4-12 mg/kg**) and the average concentration should be close to the target factory addition (e.g. **8 mg/kg**).
- Fortified sugar is packaged and labeled as required in the National Standards for General Labeling of Prepackaged Foods and the Sugar Fortification Regulations.

The *Quality Control Department* is responsible of this component, which should send daily reports to the *Factory Manager.*

[4] Based on ECSA 2007 guidelines.

II. Procedures

a. Supervision and sampling (appointed person from the QC Department)

1. Make unannounced visits to the fortification place to check that the amount of premix dispatched by the feeder has been verified; the feeder contains the vitamin A premix and is working properly, and that presence of vitamin A is being checked in hourly samples using a qualitative assay. Sign **Table B-1** and **B-2** to record completion of this supervision.
2. Ensure that personnel in the packaging site are taking 250 grams samples every hour (or every 30 minutes if conditions require a more frequently sampling) and mixing equal amounts of eight single samples to constitute shift composite samples which should be labeled with the day of sample preparation.

b. Vitamin A determination and Composite Sample Preparation

3. In the laboratory, mix well the composite samples and take 100 g sugar to determine the retinol concentration using the “Semi-quantitative method to determine retinol in fortified sugar” (**Section E**), or the “Spectrophotometric quantitative method” (**Section F**), if the necessary equipment is available.

4. Plot 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: 0-5 mg/kg, 5-10 mg/kg, 10-15 mg/kg, 15-20 mg/kg and >20 mg/kg. If the quantitative method was used write down the results in the last column intended for these results.

5. Prepare a **daily** composite sample, mixing 500 grams from each of the shift composite samples collected during the day. Mix well. Determine the content of vitamin A, and record result in **Table C-1** as *Daily comp*.

   Store the remaining daily-composite sample in an air-tight and opaque container. Identify the sample with the date, and include the result of vitamin A for this sample. Keep this sample in the sample storage room for up to a month.

6. At least every two weeks, select randomly two daily-composite samples from the sample storage room and send them to an external reference laboratory for the quantitative determination of vitamin A.

c. Corrective actions

7. If abnormalities are found, discuss immediately with the production supervisor the measures to correct them.

III. Records and Reporting

1. Complete **Table C-1** with the data provided by the production supervisor.

2. Calculate the ratio sugar produced/premix. For example, if the target vitamin content is 7.5-8.0 mg/kg, the ratio should be close to 2.0 in weight (MT sugar/kg premix) or 1000 if it is expressed in 50-kg sacks sugar over 25-kg bags premix[5].

3. Record all the needed information in **Table C-1**, and send daily a copy to the production manager for attention.

4. *Results from external laboratory:* Once results are received, record those in the last column of **Table C-1**intended for quantitative results; specify which samples were analyzed and that results come from an external laboratory. Compare the results with your own data, and if incompatibility is found look for the reason, and apply corrective measures as needed.

5. Send reports to the production manager about corrective actions or confirmation of the prior conclusions and deductions from the work of the Quality Control Department.

[5] If fortification level is 15 mg/kg, then the ratio should be 1.0 (or 500 if expressed in terms of 50 kg-bags of fortified sugar per 25-kg bags of premix).

D. FIXED CUT-OFF POINT METHOD TO DETERMINE VITAMIN A IN SUGAR (QUALITATIVE METHOD)

I. References

Calzia R, Martinez C, Dominguez P and Dary O. A cut-off point method to determine vitamin A in sugar and other foods for fast screening in monitoring programs. W35. Institute of Nutrition of Central America and Panama (INCAP/PAHO), Universidad de San Carlos de Guatemala. Presented in the XXI IVACG Meeting, Marrakech, Morocco. 3-5 February, 2003.

Bayfield, R.F. and Cole, E.R. 1980. Colorimetric determination of vitamin A with trichloroacetic acid. In: McCormick, D.B. and Wright, L.D., *Eds Methods in Enzymology, part F. Vitamins and Coenzymes*. Vol. 67. New York: Academic Press. pp 189-195.

Arroyave, G., Pineda, O. y Funes, C. de. 1974. Enriquecimiento de azúcar con vitamina A. Método rápido para la fácil inspección del proceso. *Arch. Latinoamer. Nutr*. **24**: 155-159.

II. Principle

This method is qualitative and determines the presence of retinol in sugar at a concentration around the established cutoff points of either 3.5 mg/kg or 5.0 mg/kg. The cut-off point is the minimum concentration that can be detected with relatively good selectivity.

Retinol present in sugar reacts with trichloroacetic acid to form anhydroretinol. During the reaction, a blue color can be observed indicating the presence of retinol in the sample. The blue color is transient, so if the color develops, it must be observed within 10 seconds after adding the reagent.

III. Critical Points and Cautions

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^{o}C. If crystals develop, they can be dissolved by manual agitation of the container.

The chromogenic reagent is corrosive and should be handled with care by trained personnel. Immediately before use, the volume required

should be transferred to a beaker, from which it can be drawn into a pipette before being added to the sugar solution. The reagent goes turbid in a humid environment, so it must be kept capped until needed. In addition, the beaker into which it is poured must be dry and at room temperature. After the completion of the analysis, any remaining reagent in the beaker should be discarded appropriately and NOT returned to its original container.

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IV. Equipment and Materials

- Analytical balance
- Beakers (25, 250 and 600 mL)
- Wide-mouth dark glass bottle (to collect used reagent)
- Disposable rubber gloves
- Plastic spoon
- Polyethylene pasteur pipette
- Hot plate
- Graduated cylinder (100 and 10 mL)
- Dark glass bottle with glass stopper
- Test tubes (15mm x100 mm)
- Watch glass
- Glass rod

V. Reagents

- Chromogenic reagent: Trichloroacetic acid/Dichloromethane/acetic anhydride for cut-off point -3.5 mg/kg. Mix 60.0 g trichloroacetic acid (FW: 163.39, 99.5%) with 80.0 g dichloromethane (60.6 mL) (FW: 84.93, 99.5%, d=1.32 g/mL). To dissolve completely, warm up the mixture in a water bath at 50°C stirring constantly. Add 2 mL of acetic anhydride (FW: 102.092) and store in a dark bottle with glass stopper, preferably in a refrigerator.

Table (1) below shows the amount of reagents needed for both cut-off point 3.5 mg/kg and 5 mg/kg.

TABLE -1. CHROMOGENIC REAGENT COMPOSITION DEPENDING ON THE CUT-OFF POINT

Reagent	Retinol cut-off point	
	3.5 mg/Kg	5 mg/Kg
Trichloroacetic acid	60 g	30 g
Dichloromethane	80 g (60.6 mL)	80 g (60.6 mL)
Acetic anhydride	2 mL	2 mL

VI. Procedure

1. Homogenize the sugar samples within their bags, with gentle rotary movements.

2. In a test tube, place about 1-g sugar measured with a plastic spoon.

3. Add 2 mL distilled water at 35° – 40 °C and dissolve the sugar.

4. Using a polyethylene Pasteur pipette, add 0.6 mL chromogenic reagent. Write down the result as positive (+) or negative (-) only. If the result is positive, that is retinol is present in levels around the fixed cut-off point, a blue color will be observed.

5. The result is positive when the color of the solution turns blue or light blue after the addition of the chromogenic agent. The intensity of the color will vary depending on the concentration of vitamin A in the sample. When vitamin A concentration is low, a few sugar crystals will turn light blue and deposit at the bottom of the tube slowly. Register this result as positive.

6. When the light blue color is barely visible or no change in color is observed, the result is negative.

7. Discard residual chromogenic reagent, including the sugar-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.

8. After the bottle is filled, the content can be discarded appropriately as other organic waste material, burning it in a chemical incinerator equipped with an afterburner and scrubber.

E. SEMI-QUANTITATIVE METHOD TO DETERMINE VITAMIN A IN FORTIFIED SUGAR

I. References

Arroyave, G., O. Pineda, and C. de Funes, (1974). Enriquecimiento de Azúcar con Vitamina A. Método Rápido para la Fácil Inspección del Proceso. *Arch. Latinoamer. Nutr.* 24: 155-159.

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.

II. Principle

The method described here is a modification of that proposed by Arroyave, Pineda, and Funes (1974). This method is based on the formation of anhydroretinol when retinol is mixed with a chromogenic reagent prepared with trichloroacetic acid dissolved in dichloromethane. A blue complex is formed and the intensity of the color can be measured semi-quantitatively by visual comparison against a reference scale of copper sulfate solutions. The blue color is transient, so the comparison should be done within 10 seconds of adding the reagent.

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 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 sugar 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. 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 the sugar solution. A syringe rather than a pipette is used because the addition of the reagent should be vigorous and rapid. The reagent goes turbid in a humid environment, so it must be kept capped until needed. 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
- Beaker (50-100mL)
- Colorimetric scale of copper sulfate solutions
- Disposable rubber gloves
- Glass test tubes (15mm x 100mm)
- Plastic bottle (50mL)
- Wide mouth glass bottle (to collect used reagent)
- Water bath (50-60°C)
- Bottle (500mL) or thermos (for distilled water)
- Dark glass bottle with glass stopper
- Glass syringe (5-10mL) with 3 cm teflon[1] tip
- Graduated pipettes (10 mL)
- Watch glass

V. Reagents

Chromogenic reagent: Trichloroacetic acid/Dichloromethane/acetic anhydride Mix 120.0 g trichloroacetic acid (FW: 163.39, 99.5%) with 80.0 g dichloromethane (60.6 mL) (FW: 84.93, 99.5%, d=1.32 g/mL). To dissolve completely, warm the mixture in a water bath at 50°C stirring constantly. Add 2 mL of acetic anhydride (FW: 102.092) and store in a dark bottle with glass stopper, preferably in a refrigerator. 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 ($CuSO_4 \bullet 5H_2O$).

Volume (mL) $CuSO_4 \bullet 5H_2O$-300 g/L to prepare 10 mL	$[CuSO_4 \bullet 5H_2O]$ (g/L)	Equivalent Retinol concentration (mg/kg)
1	30	5
2	60	10
3	90	15
4	120	20

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

Measure 5 mL each of the copper sulfate standard solutions into the same type of tubes 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. Identify each tube with its number, indicating the **concentration of retinol in mg/kg** that the color represents. These solutions are stable and can be kept indefinitely at room temperature.

VI. Procedure

a. Solubilizing vitamin A from the fortified sugar

1. Mix the sugar sample thoroughly.
2. In a 250-mL beaker, weigh 50-g sugar.
3. Add 50-mL distilled water at 50-60°C. Dissolve the sugar heating the solution if necessary.
4. Cool the solution to room temperature.

b. Preparing for colorimetric reaction

5. Transfer 1-mL sugar solution to a test tube (a tube previously marked at a 1-mL level can be used).
6. Decant enough chromogenic reagents for all the samples into a clean glass beaker.
7. Wearing disposable rubber gloves, add 3 mL of chromogenic reagent to the sugar sample solution in the test tube using a syringe. Mix immediately and vigorously.
8. Compare the intensity of the blue color of the samples with the copper sulfate standards within 10 seconds of adding the reagent, because the color change is transient.
9. Estimate the approximate concentration of retinol in the sugar sample (mg/kg) 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 the sugar should be reported as falling within the range corresponding to the reference tubes. For example, if the blue intensity of the sample solution lies somewhere between the levels of 30 and 60 g/L copper sulfate standard solutions, the retinol level is between 5 and 10 mg/kg.

c. Discharging the used reagents

10. Discard residual chromogenic reagent, including the sugar-reagent mixture, into a glass bottle containing dissolved sodium bicarbonate, slowly adding the reagent to the bottle. The bottle should be clearly labeled asa waste bottle.
11. After the bottle is filled, the content can be discarded appropriately as other organic waste material, burning it in a chemical incinerator equipped with an afterburner and scrubber.

F. QUANTITATIVE SPECTROPHOTOMETRIC METHOD TO DETERMINE VITAMIN A IN FORTIFIED SUGAR

I. References

Arroyave G. and Funes C. de (1974) Enriquecimiento de Azúcar con Vitamina A. Método para la Determinación Cuantitativa de Retinol en Azúcar Blanca de Mesa. *Arch. Latinoamer. Nutr.* **24**:147-153.

II. Principle

This method is an adaptation of the method developed by Arroyave and Funes (1974). The procedure uses five to ten times less reagent volume than the original method, and its accuracy is similar. In the past, 20 g sugar sample were used and it has been increased to 100 g in order to improve the precision and reproducibility of the analysis.

Sugar sample is dissolved in warm water to dissolve the matrix of vitamin A fortificant compound. The sugar solution is diluted 1:2 with sodium hydroxide and then, the released vitamin A (as retinyl palmitate) is extracted into hexane. Retinyl palmitate concentration is determined by the absorbance of this solution at 326 nm. This method does not usually require irradiation with UV light, because the absorbance of the extract at 326 nm is mainly due to the retinyl palmitate in sugar.

III. Critical Points and Cautions

A spectrophotometer capable of reading 326 nm is essential. This is because the concentration of the retinyl palmitate standards is measured by spectrophotometric analysis. Given the importance of the spectrophotometer for ensuring the accuracy and reliability of the vitamin A 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.

It is critical that water used to dissolve the sample is 85° C to assure the matrix of the vitamin A compound dissolves completely. Once retinyl palmitate has been extracted in hexane, the analysis should not be interrupted. Based on the experience at the laboratory of the Institute of Nutrition of Central America and Panama (INCAP), if the variability between replicates of the same solution is greater than 5%, the results should be rejected and the extractions repeated. The recovery of the method is at least 91%.

IV. Equipment and Materials

- UV Spectrophotometer (326 nm)
- Beaker (250 mL)
- Aspiration bulbs for Pasteur pipettes and graduate pipettes
- Black clothing
- Pasteur pipettes
- Spectrophotometer quartz cuvettes (UV)
- Volumetric flasks (200-250 mL)
- Vortex mixer
- Test tubes with screw caps (50 mL)
- Graduated cylinder (100 mL)
- Glass rods
- Spatulas
- Test tube rack
- Volumetric or serologic pipettes (to measure 2, 3 and 8 mL)

V. Reagents

- Absolute ethanol (C_2H_5OH), AR grade, purity=99.8%, FW=46.07, d=0.79 g/mL
- Phenolphthalein solution-1% m/v in ethanol; Phenolphthalein ($C_{20}H_{14}O4$), FW=318.33
- Hexane (C_6H_{14}), AR grade, purity=99%, FW=86.18, d=0.66 g/mL
- Sodium hydroxide solution-0.1 N; Sodium hydroxide (NaOH), purity=97%, FW=40.00

VI. Procedure

a. Solubilizing vitamin A from the fortified sugar

1. Homogenize the sample inside the container with gentle rotary movements.
2. Weigh approximately 100 g of sugar, recording the exact weights to two decimal places; place the sugar in a 250-mL beaker and add about 100 mL hot water at 85°C. Use a glass rod to completely dissolve the sample. Cover the beakers with a watch glass or aluminum foil.[1]
3. Cool them to room temperature in a dark place. An ice bath can be used for this purpose.
4. Transfer to a 250 mL volumetric flask. Rinse the beaker with small amounts of distilled water and transfer the washings to the volumetric flask. Make up to 250 mL with distilled water and mix.
5. If samples are expected with vitamin A levels above 20 mg/kg, dilute the sugar solution 1:1 with water (same amounts of the sugar solution and water) before proceeding to the following step. This step is going to introduce an additional dilution factor of 2 for the samples with higher content of vitamin A.

b. Extracting vitamin A from the fortified sugar

6. Measure 10 mL of the solution prepared in steps (4 or 5) into a 50 mL test tube. Prepare triplicates for each sample.

7. Add 5 mL of 0.1 N-sodium hydroxide to each tube and mix in a Vortex for 30 seconds.

8. Add 2-3 drops phenolphtalein-1% m/v to the same tubes. Then, add 5 mL absolute ethanol to each tube. Mix in the vortex mixer for 5 seconds.

1 This procedure should dissolve sugar and vitamin A premix for most samples. Sometimes samples are difficult to dissolve and they need further heating. In this case, place the beakers in a water bath at 55-65°C for 10-15 minutes.

9. Measure 5 mL of hexane and add it to each tube from step (8). Immediately close with a cap each tube and mix vigorously with the vortex mixer for 30 seconds to ensure complete extraction of the retinyl palmitate.

Open the tubes briefly to release the vapor pressure. Allow separation of phases. The aqueous phase has a fuchsia color, and the top organic solvent phase is colorless.

c. Recording absorbance of the extracted vitamin A

10. As soon as possible, transfer the organic phase, using a Pasteur pipette to a 1 cm light path spectrophotometer cuvette and read the absorbance at 326 nm. Adjust the zero of spectrophotometer with hexane before each reading.

VII. Calculations

The retinyl palmitate concentration of the sugar sample is calculated using the following equation:

$$\text{Retinyl palmitate } (mg/kg) = \frac{Abs_{corrected}}{a} \times \frac{V_{org}}{V_{aliq.}} \times \frac{V_t}{w} \times \frac{CF_{spec}}{R} \times D$$

Where:
$Abs_{corrected} = Abs_{sample} - Abs_{blank}$
And Abs_{blank} is the average for the three readings, which should be less than 0.050

The equation parameters are:

PARAMETER	EXPLANATION	VALUE
a	Retinyl palmitate absorption coefficient in hexane (mg^{-1} cm^{-1} L)..... .	0.092
V_{org}	Volume of the organic phase (mL)	5.0
V_{sugar}	Volume of the aliquot analyzed from the sugar solution (mL)	10.0
V_I	Volume of the initial solution of the sample (mL).....	250.0
w	Weight of the sample (g)	data from weight
R	Recovery	0.905
CF_{spec}	Correction factor of the spectrophotometer. Ideally...	1
D	Dilution Factor from mixing with NaOH (10/5)	2

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. Simplified equations to estimate the unesterified retinol are:

a. For samples without step (5)

$$Retinol\ (mg/kg) = Abs_{corrected}\ x\frac{1639.4}{w}xCF_{spec}$$

b. For samples with step (5): an additional dilution 1:2:

$$Retinol\ (mg/kg) = Abs_{corrected}\ x\frac{3278.8}{w}xCF_{spec}$$

FORTIFIED SUGAR QC/QA - TABLE A-1
VITAMIN A PREMIX INVENTORY CONTROL LOG

Harvest year:_____ Page No. _____

DATE	TIME	RECEIVED			DISPATCHED		IN STOCK (C) (C) = (A) – (B)	OBSERVATIONS/ QC-Review (Name and signature)
		No. BAGS (A)	LOT ID (BAG Nos.)	PROD. DATE	No. BAGS (B)	LOT ID (BAG Nos.)		

Samples send to laboratory:	Identification:	[Vit.A] =	Identification:	[Vit.A] =

Date of reporting: _____ Name and signature: _____

FORTIFIED SUGAR QC/QA - TABLE B-1

FEEDER FLOW CONTROL FOR PRODUCTION OF SUGAR FORTIFIED WITH VITAMIN A[1]

Sugar factory: _____ **Page No.** _____

Harvest year: _____ **Feeder location:** _____

DATE	SHIFT (TIME)	PRODUCTION RATE (MT/HOUR)	THEORETICAL FEEDER FLOW (g/min)[2]	FEEDER FLOW (g/min)				ADJUSTED (YES/NO)	RESPONSIBLE	OBSERVATIONS/ QC-Review (Name and signature)
				1	2	3	Mean			

[1] This table should be kept near to the feeder site. Once a sheet is full, it should be sent to the Quality Control Department.
[2] Feeder flow (g/min) = MT/hour x 100/6

FORTIFIED SUGAR QC/QA - TABLE B-2
PRODUCTION LOG FOR SUGAR FORTIFIED WITH VITAMIN A

Sugar factory: _____ **Page No.** _____

Harvest year: _____ **Feeder location:** _____

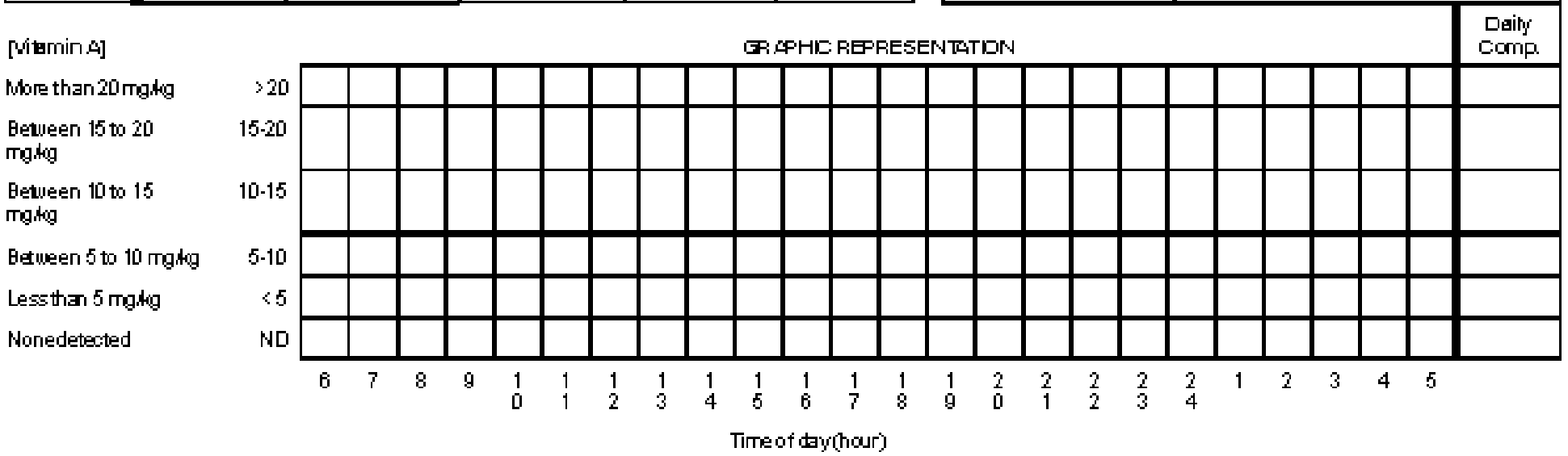
SHIFT (TIME)	SUGAR PRODUCED	PREMIX USED		SUGAR/PREMIX RATIO (A/B)	RESPONSIBLE	OBSERVATIONS/ QC-Review (Name and signature)
	No. 50-kg BAGS (A)	No. 25-kg BAGS (B)	LOT ID			

FORTIFIED SUGAR QC/QA - TABLE C-1

PRODUCTION AND QUALITY CONTROL LOG FOR FORTIFIED SUGAR[1] WITH VITAMIN A

SHIFT (Time)	SUGAR PRODUCED # 50-kg sacks	PREMIX USED # 25-kg bags	FORTIFIED SUGAR (M.T.)	PREMIX USED (kg)	SUGAR/ PREMIX
Daily Total					
Total To Date					

COMMENTS:	DATE:
	Results from Quantitative Testing[2]:
	[Vitamin A] (mg/kg) =
Responsible:	Signature:



[1] This table is based on Log-form from the Los Tarros Refinery, S.A. in Guatemala

[2] These results may be obtained in the factory quality control laboratory or from an external laboratory.

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