

# Dried skimmed milk — Determination of vitamin A content

## Part 1: Colorimetric method

ICS 67.100.10

## National foreword

This British Standard is the UK implementation of ISO 12080-1:2009. It supersedes BS ISO 12080-1:2000 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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**Dried skimmed milk — Determination of  
vitamin A content —**

**Part 1:  
Colorimetric method**

*Lait écrémé en poudre — Détermination de la teneur en vitamine A —  
Partie 1: Méthode colorimétrique*



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## Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 12080-1|IDF 142-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

ISO 12080|IDF 142 consists of the following parts, under the general title *Dried skimmed milk — Determination of vitamin A content*:

- *Part 1: Colorimetric method*
- *Part 2: Method using high-performance liquid chromatography*

This second edition of ISO 12080-1|IDF 142-1 cancels and replaces the first edition (ISO 12080-1:2000), of which it constitutes a minor revision.

## Foreword

**IDF (the International Dairy Federation)** is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

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ISO 12080-1|IDF 142-1 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the former Joint ISO-IDF Group of Experts (E46 — *Vitamins A and D in dried milk*) which is now part of the Joint ISO-IDF Action Team on *Organic contaminants and veterinary residues* of the Standing Committee on *Analytical methods for additives and contaminants*.

ISO 12080|IDF 142 consists of the following parts, under the general title *Dried skimmed milk — Determination of vitamin A content*:

- *Part 1: Colorimetric method*
- *Part 2: Method using high-performance liquid chromatography*

This edition of ISO 12080-1|IDF 142-1, together with ISO 12080-2|IDF 142-2, cancels and replaces IDF 142:1990, of which it constitutes a minor revision.

## Introduction

The methods specified in ISO 12080|IDF 142 (all parts) have been selected after consideration and laboratory testing of a variety of alternative procedures. Their advantages include the absence of highly dangerous reagents as in, for example, the Carr-Price method, and the avoidance of reagents that are not universally available.

The decision to provide two separate methods was taken to meet the needs both of laboratories with sophisticated equipment (HPLC) and those without such apparatus.

Although the International Standard for vitamin A was discontinued in 1954, the International Unit for this substance has continued to be widely used and its use has been maintained in this International Standard. The International Unit for vitamin A was redefined in 1960 as the activity of 0,344 µg of pure all-*trans*-vitamin A acetate (see Annex A).





# Dried skimmed milk — Determination of vitamin A content —

## Part 1: Colorimetric method

**WARNING** — The use of this International Standard may involve hazardous materials, operations and equipment. Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish health and safety practices and determine the applicability of regulatory limitations prior to use.

### 1 Scope

This part of ISO 12080|IDF 142 specifies a colorimetric method for the determination of vitamin A in dried skimmed milk containing at least 10 IU (International Units) of vitamin A per gram.

### 2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

#### 2.1

##### **vitamin A content of dried skimmed milk**

mass fraction of substances determined by the procedure specified in this part of ISO 12080|IDF 142

**NOTE** Vitamin A content is expressed either in micrograms of retinol per gram or in International Units of vitamin A activity per gram.

### 3 Principle

The test sample is saponified and extracted. The unsaponifiable matter is reacted with trifluoroacetic acid. The absorbance at 620 nm is measured.

### 4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

**4.1 Ethanol** (CH<sub>3</sub>CH<sub>2</sub>OH), 95 % volume fraction, free from aldehyde.

**4.2 Sodium ascorbate solution**, 200 g/l. If not available ready-made, prepare by dissolving 3,5 g of ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>) in 20 ml of 1 mol/l sodium hydroxide (NaOH) solution and mix. Prepare this solution fresh daily.

**4.3 Potassium hydroxide aqueous solution** (KOH), 50 % mass fraction. Dissolve 50 g of potassium hydroxide in 50 ml of water. Mix and cool the solution. Prepare this solution just before use.

**4.4 Potassium hydroxide aqueous alcoholic solution**, 30 g/l. Dissolve 3 g of potassium hydroxide (KOH) in water and add 10 ml of ethanol (4.1) in a 100 ml one-mark volumetric flask. Make up to the mark with water and mix. Prepare this solution just before use.

**4.5 Light petroleum**, with a boiling range between 40 °C and 60 °C, or between 60 °C and 80 °C.

**4.6 Chloroform** (CHCl<sub>3</sub>).

**4.7 Trifluoroacetic acid** (CF<sub>3</sub>COOH).

**WARNING — Chloroform and trifluoroacetic acid are carcinogenic. Take all necessary precautions.**

**4.8 Colour reagent.** Mix one volume of pure trifluoroacetic acid (4.7) with two volumes of chloroform (4.6).

**4.9 Vitamin A standard solution.** Use US Pharmacopeia<sup>1)</sup> standard reference solution of vitamin A made from crystalline all-*trans*-retinyl acetate in cottonseed oil, equivalent to 30 mg of retinol (vitamin A alcohol, C<sub>20</sub>H<sub>30</sub>O) per gram of oil, or as stated when purchased.

A secondary standard solution may be used if standardized against this primary standard reference solution or by UV measurement.

Cut the tip from the capsule containing the vitamin A standard reference solution and express the oil into a (tared) 100 ml amber-coloured one-mark volumetric flask. Weigh the contents to the nearest 0,1 mg. Make up to the mark with chloroform (4.6). Use the vitamin A standard solution as soon as possible. Discard the solution after 8 h.

**4.10 Butylated hydroxytoluene** (BHT).

## 5 Apparatus

Usual laboratory apparatus and, in particular, the following.

**5.1 Photoelectric colorimeter or spectrometer**, with an optical mechanism or filter for a wavelength of 620 nm (vitamin A derivative).

Use matched absorption cells. An instrument providing linearity between absorbance and concentration is to be preferred.

**5.2 Beaker or conical flask**, of capacity 250 ml.

**5.3 Saponification flask**, of capacity approximately 200 ml, fitted with a reflux condenser.

**5.4 One-mark volumetric flasks**, of capacities 100 ml and 200 ml, ISO 1042<sup>[3]</sup> class A.

**5.5 One-mark pipettes**, of capacities 2 ml, 10 ml, 25 ml and 50 ml, ISO 648<sup>[1]</sup> class A.

**5.6 Automatic pipettes**, suitable for organic solvents, ISO 8655-2<sup>[6]</sup>, or a **pipette**, to deliver 10 ml.

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1) Example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 12080|IDF 142 and does not constitute an endorsement by ISO or IDF of this product.

**5.7 Steam bath, boiling water bath or electric heating mantle.**

**5.8 Water bath**, capable of operating at a temperature of up to 40 °C.

**5.9 Separating funnel**, of capacity 500 ml, preferably with a polytetrafluoroethylene (PTFE) stopper.

**5.10 Ultrasonic bath.**

**5.11 Filter paper**, of diameter 90 mm.

## 6 Sampling

Sampling is not part of the method specified in this part of ISO 12080|IDF 142. A recommended sampling method is given in ISO 707|IDF 50<sup>[2]</sup>.

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

## 7 Preparation of test sample

Thoroughly mix the test sample by repeatedly rotating and inverting the sample container. If necessary, transfer the complete test sample to an airtight container of sufficient capacity.

## 8 Procedure

### 8.1 General

If a check is required of whether the repeatability limit (10.2) is met, carry out two single determinations in accordance with 8.2 to 8.5.

For all operations, work in subdued light or use low-actinic glassware.

### 8.2 Test solution

Weigh, to the nearest 0,001 g, about 20 g of the test sample into a beaker or conical flask (5.2) and dissolve in 50 ml of water at a temperature of least 80 °C. Break up any lumps with a spatula or by using an ultrasonic bath (5.10). Cool to room temperature. Transfer quantitatively to a 100 ml one-mark volumetric flask (5.4). Make up to the mark with water.

### 8.3 Saponification and extraction

**8.3.1** Transfer, by means of a pipette (5.5), 25 ml of the prepared test solution (8.2) to a saponification flask (5.3). Add 20 ml of potassium hydroxide solution (4.3) and 10 ml of sodium ascorbate solution (4.2). Add 50 ml of ethanol (4.1) and mix well.

**8.3.2** Reflux for 30 min on a steam bath (5.7), swirling from time to time. Cool immediately under running water.

**8.3.3** Transfer the liquid to a separating funnel (5.9), and wash twice, each time using 30 ml of water, 10 ml of ethanol (4.1), and 40 ml of light petroleum (4.5). Shake vigorously for 30 s and allow to stand until the two layers are clear.

Transfer the aqueous (lower) phase to a second separating funnel and shake with a mixture of 10 ml of ethanol (4.1) and 40 ml of light petroleum (4.5). Leave to separate.

**8.3.4** Transfer the aqueous phase to a third separating funnel and the light petroleum phase to the first separating funnel. Wash the second separating funnel twice with 10 ml of light petroleum (4.5). Add the washings to the first separating funnel.

**8.3.5** Shake the aqueous phase with 40 ml of light petroleum (4.5) and 10 ml of ethanol (4.1). Add the light petroleum phase to the first separating funnel. Wash the combined light petroleum extracts three times with 40 ml freshly prepared potassium hydroxide aqueous alcoholic solution (4.4), shaking vigorously. Then wash with 40 ml volumes of water until the last washing is neutral to phenolphthalein. Drain the last few drops of water, add two sheets of filter paper (5.11), cut into strips, to the separating funnel and shake.

**8.3.6** Transfer the light petroleum extract from which water has been removed (8.3.5) to a 200 ml one-mark volumetric flask (5.4). Rinse the separating funnel and paper with light petroleum (4.5), add the rinsings to the volumetric flask, then add 10 mg to 20 mg of BHT (4.10). Make up to the mark with light petroleum and mix well.

## 8.4 Preparation of test colorimetric solution

Pipette an aliquot of the diluted extract (8.3.6) into a round-bottom flask. Evaporate to dryness under vacuum by swirling in a water bath (5.8) at a temperature not exceeding 40 °C. Cool under running water and restore atmospheric pressure, preferably with nitrogen. Dissolve the residue immediately in 10,0 ml of chloroform (4.6).

NOTE A typical concentration in the test solution is 10 IU of vitamin A per millilitre of chloroform. The amount of the aliquot can be adjusted depending on the size and sensitivity of the colorimetric cell.

## 8.5 Determination

Designate two suitable matched colorimetric cells as 1 and 2. Pipette 2 ml of the test colorimetric solution (8.4) into cell 1. Pipette 2 ml of the diluted vitamin A standard solution (8.6) into cell 2. Rapidly add to each cell, preferably using an automatic pipette (5.6), 10,0 ml of the colour reagent (4.8) and mix. Monitor the absorbance of the solutions at 620 nm with the spectrometer or photoelectric colorimeter (5.1), measured against a blank of 2 ml of chloroform (4.6) and 10 ml of colour reagent (4.8), until the absorbance reaches its maximum. Plot a graph of the obtained absorbance against the vitamin A content (8.6).

The volumes and proportions used may be adjusted proportionally according to the capacity of the colorimeter cells.

## 8.6 Preparation of calibration graph

Make five-fold dilutions (or greater) of the vitamin A standard solution (4.9) with chloroform (4.6) so that 2 ml aliquot portions treated in the colorimetric determination give absorbances in the range of 0,07 to 0,7 at 620 nm. Plot absorbance against the mass, in micrograms, of vitamin A. If the graph is a straight line, a factor for the content of vitamin A in the samples can be calculated.

## 9 Calculation and expression of results

Calculate the vitamin A content,  $w$ , in micrograms of retinol per gram (or the vitamin A activity, expressed in International Units per gram), using the equation:

$$w = \frac{\rho V_1 V_3 V_4}{V_2 V_5 m}$$

where

- $\rho$  is the concentration, in micrograms of retinol per millilitre (or vitamin A activity in IU per millilitre), in the test colorimetric solution (8.4), calculated from the calibration graph (8.6);
- $V_1$  is the total volume, in millilitres, of light petroleum extract ( $V_1 = 200$  ml);
- $V_2$  is the volume, in millilitres, of the aliquot taken from  $V_1$  (8.4);
- $V_3$  is the volume, in millilitres, of chloroform in which the residue is dissolved ( $V_3 = 10$  ml);
- $V_4$  is the total volume, in millilitres, of the test solution (8.2) ( $V_4 = 100$  ml);
- $V_5$  is the volume, in millilitres, of the aliquot part of the test solution (8.3.1) ( $V_5 = 25$  ml);
- $m$  is the mass, in grams, of the test portion (8.2).

## 10 Precision

### 10.1 Interlaboratory test

Details of an interlaboratory test carried out in accordance with ISO 5725-1<sup>[4]</sup> and ISO 5725-2<sup>[5]</sup> on the precision of the method have been published (Reference [7]). The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

### 10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, shall in not more than 5 % of cases be greater than 14 % of the arithmetic mean of the two results.

### 10.3 Reproducibility

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, shall in not more than 5 % of cases be greater than 42 % of the arithmetic mean of the results.

## 11 Test report

The test report shall contain at least the following information:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, together with reference to this part of ISO 12080 | IDF 142;
- d) all operating details not specified in this part of ISO 12080 | IDF 142, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the test result(s) obtained, or, if the repeatability has been checked, the final result obtained.

## Annex A (informative)

### Activity expressed in International Units (IU)

#### A.1 Activity of vitamin A

The activity of vitamin A is expressed in International Units. It has been defined (see Reference [8]) that 1 IU of vitamin A corresponds to the activity of 0,344 µg of all-*trans*-retinyl acetate.

The activity of the other vitamin A compounds is calculated stoichiometrically so that 1 IU corresponds to the activity of 0,300 µg of all-*trans*-retinol, 0,359 µg of all-*trans*-retinyl propionate or 0,500 µg of all-*trans*-retinyl palmitate, respectively.

This means that the activity of 1 g of pure all-*trans*-vitamin A alcohol and ester, expressed in International Units, is equal to:

— vitamin A alcohol (retinol)	3 333 000 IU;
— vitamin A acetate	2 907 000 IU;
— vitamin A propionate	2 785 000 IU;
— vitamin A palmitate	1 818 000 IU.

#### A.2 Assay of vitamin A standard (vitamin A ester, pure or dissolved in oil)

See Reference [8].

Weigh, to an accuracy of 0,1 %, 25 mg to 100 mg of vitamin A ester in a flask. Dissolve this in 5 ml of pentane and dilute, depending on the weighed amount, with 2-propanol to a presumed concentration of 10 IU/ml to 15 IU/ml.

Verify that the absorption maximum,  $A_m$ , of the solution lies between 325 nm and 327 nm using 2-propanol as compensation liquid (blank). Measure the absorbance,  $A_n$ , at 300 nm, 326 nm, and 370 nm.

Calculate the ratio  $A_n/A_m$  for each of the wavelengths mentioned. If the ratios do not exceed 0,593 at 300 nm, 0,537 at 350 nm or 0,142 at 370 nm, respectively, then calculate the vitamin A content,  $w$ , in International Units per gram, using the equation:

$$w = \frac{A_m V f}{100 \times m}$$

where

$A_m$  is the numerical value of the maximum absorbance obtained at 326 nm;

$V$  is the total volume, in millilitres, to which the vitamin A ester has been diluted to give 10 IU/ml to 15 IU/ml;

*f* is the factor to convert the specific absorbance of vitamin A esters into International Units per gram ( $f = 1\,900$ );

*m* is the mass, in grams, of the weighed vitamin A esters.

## Bibliography

- [1] ISO 648, *Laboratory glassware — Single-volume pipettes*
- [2] ISO 707|IDF 50, *Milk and milk products — Guidance on sampling*
- [3] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*
- [4] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [5] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [6] ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*
- [7] DE VRIES, E.J. et al. Dried skimmed milk — Determination of vitamin A — Colorimetric and liquid chromatographic methods, pp. 53-64. In: *Reference materials and interlaboratory collaborative studies (third series)*. [Bull. IDF 1993, (285)]
- [8] Vitamin A monograph. In: *European Pharmacopoeia*





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