

DRAFT EAST AFRICAN STANDARD

Food grade saccharin — Specification

EAST AFRICAN COMMUNITY

Copyright notice

This EAC document is copyright-protected by EAC. While the reproduction of this document by participants in the EAC standards development process is permitted without prior permission from EAC, neither this document nor any extract from it may be reproduced, stored or transmitted in any form for any other purpose without prior written permission from EAC.

Requests for permission to reproduce this document for the purpose of selling it should be addressed as shown below or to EAC's member body in the country of the requester:

© East African Community 2019 — All rights reserved
East African Community
P.O. Box 1096,
Arusha
Tanzania
Tel: + 255 27 2162100
Fax: + 255 27 2162190
E-mail: eac@eachq.org
Web: www.eac-quality.net

Reproduction for sales purposes may be subject to royalty payments or a licensing agreement. Violators may be prosecuted.

Contents

Page

Foreword.....	
1 Scope.....	
2 Normative references.....	
3 Terms and definitions.....	
4 Requirements.....	
4.1 General requirements.....	
4.2 Specific requirements.....	
5 Hygiene.....	
6 Contaminants.....	
7 Packaging.....	
8 Weights and measures.....	
9 Labelling.....	
10 Sampling.....	
Annex A (normative) Assay: Test for purity.....	
Annex B (normative) Loss on drying.....	
Annex C (normative) Test for acidity and alkalinity.....	
Annex D (normative) Determination of the melting range.....	
Annex E (normative) Test for benzoic and salicylic acid.....	
Annex F (normative) Test for readily carbonizable substances.....	
Annex G (normative) Test for Selenium.....	
Annex H (normative) Test for toluene sulfonamides.....	
Bibliography.....	

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the Principles and procedures for development of East African Standards.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

The committee responsible for this document is Technical Committee EASC/TC 005, *Food additives*.

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

Introduction

Saccharin (1,2-Benzisothiazole-3(2H)-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide) and its sodium, calcium and potassium salts are widely utilised in the food industry as non-nutritive artificial sweeteners. They are used as a substitute for cane sugar in the production of diet and low-calorie foods. They are colourless crystals or white crystalline powders with faint aroma and about 300 – 500 times sweeter than sucrose (table sugar). In the Codex Alimentarius Commission International Numbering System, saccharins are assigned as INS 954 and the FAO/WHO Joint Experts Committee on Food Additives (JECFA) established the Acceptable Daily Intake (ADI) for saccharin and its salts at 0 mg/kg – 5 mg/kg body weight.

There are two main approaches to making saccharin: the Remsen-Fahlberg process (named after the two scientists who discovered the compound) and the Maumee or Sherwin-Williams process.

The Remsen-Fahlberg process requires reacting toluene, which has a natural sweet smell, with chlorosulfonic acid, which is a colourless liquid. This acid compound is then reacted with a series of compounds, including potassium permanganate and ammonia and heated to yield saccharin. Because this process takes a lot of compounds to produce a relatively low yield, improvements were sought-which is why the Maumee process was created.

The Maumee process begins with converting phthalic anhydride, an industrial compound used in creating plastics to anthranilic acid. The acid is reacted with several compounds, including nitrous acid, sulphur dioxide, chlorine and ammonia to produce saccharin.

Common food products containing saccharin and/or its Na, Ca or K salts include: soft drinks (sodas), low-calorie jams or jellies, biscuits, candies and dessert toppings such as flavoured syrups for ice cream, and salad dressings. These sweeteners are also used in pharmaceutical and oral hygiene products.

This standard has been developed to ensure that the saccharin used in the food industry is safe for human consumption.

Food grade saccharin — Specification

1 Scope

This Draft East African Standard specifies requirements, sampling and test methods for food grade saccharin intended for use in food products.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

AOAC 952.13, *Arsenic in food. Silver diethyldithiocarbamate method*

AOAC 972.25, *Lead in food. Atomic absorption spectrophotometric method*

CAC/GL 50, *General guidelines on sampling*

CODEX STAN 107, *General standard for the labelling of food additives when sold as such*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

food grade material

material, made of substances that are safe and suitable for their intended use and which will not impart any toxic substance or undesirable odour or flavour to the product

3.2

artificial sweetener

synthetic substance used as a sugar substitute to sweeten food and drink

4 Requirements

4.1 General requirements

Food grade saccharin shall be:

- a) white crystals or white crystalline powder;
- b) odourless or with a faint aromatic odour; and
- c) slightly soluble in water, soluble in basic solutions and sparingly soluble in ethanol. 1 g of product shall be soluble in 1.5 ml of water or basic solutions and in about 50 ml of ethanol.

4.2 Specific requirements

Food grade saccharin shall comply with the specific requirements given in Table 1 when tested in accordance with the test methods specified therein.

Table 1 — Specific requirements for food grade saccharin

S/N	Characteristic	Requirement	Test method
i)	Percentage purity, as $C_7H_5NO_3S$, % m/m (dry matter basis)	99 – 101	Annex A
ii)	Loss on drying, % m/m, max.	1	Annex B
iii)	Acidity and alkalinity at 25 °C	To pass test	Annex C
iv)	Melting range, °C	226 – 230	Annex D
v)	Benzoic and salicylic acid	To pass test	Annex E
vi)	Readily carbonizable substances	To pass test	Annex F

5 Hygiene

Food grade saccharin shall be manufactured and handled in accordance with EAS 39.

6 Contaminants

Food grade saccharin shall comply with the maximum levels of contaminants given in Table 2 when tested in accordance with the test methods specified therein.

Table 2 — Maximum Limits for contaminants in food grade saccharin

S/N	Contaminant	Maximum level mg/kg	Test method
i)	Lead (Pb)	1	AOAC 972.25
ii)	Arsenic (As)	2	AOAC 952.13
iii)	Selenium (Se)	30	Annex G
iv)	Toluenesulfonamides	25	Annex H

7 Packaging

Food grade saccharin shall be packaged in clean, sound and airtight food grade materials. The packages shall preserve the quality and safety of the product and preclude contamination from the external environment.

8 Weights and measures

The products shall comply with the Weights and Measures Regulations of the respective Partner States

9 Labelling

In addition to the requirements of CODEX STAN 107, the product packages shall be legibly and indelibly labelled with the following information:

- a) name of the product as “Food grade saccharin”;
- b) name and physical address of the processor/packer/importer;
- c) date of manufacture;
- d) expiry date; and
- e) net weight of the product in metric units.

10 Sampling

Representative samples of the product shall be drawn in accordance with CAC/GL 50.

Annex A

(normative)

Assay: Test for purity

A.1 Apparatus

A.1.1 Analytical balance

A.1.2 Hot air oven

A.2 Reagents

A.2.1 0.1N sodium hydroxide

A.2.2 Phenolphthalein indicator

A.2.3 Distilled water

A.3 Procedure

Dry 10 g of product in a hot air oven at 120 °C for 4h. Accurately weigh about 0.5 g (M) of the dried sample and dissolve it in 75 ml of hot water. Quickly cool the mixture and add two to three drops of phenolphthalein indicator. Titrate the mixture with 0.1N sodium hydroxide till the endpoint and record the volume of NaOH used (V).

Each millilitre of 0.1N sodium hydroxide is equivalent to 18.32 mg of $C_7H_5NO_3S$.

The purity (as $C_7H_5NO_3S$), expressed as percentage m/m (dry matter basis), shall be calculated as follows:

$$\frac{18.32 \times V}{M \times 1000} \times 100$$

where

V is the volume, in millilitres, of 0.1N NaOH used and

M is the mass, in grams, of dried sample dissolved in 75 ml of hot water.

Annex B

(normative)

Loss on drying

B.1 Apparatus

- B.1.1 Weighing bottle with a stopper
- B.1.2 Air oven
- B.1.3 Desiccator
- B.1.4 Weighing scale

B.2 Sample preparation

Weigh 1 to 2 g of sample (M_1). Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 minutes at 105 °C. Transfer the sample into the bottle, replace the cover, and weigh the bottle and the sample (M_2). Distribute the sample as evenly as practicable to a depth of about 5 mm, and not over 10 mm.

B.3 Procedure

Place the bottle with its contents in the drying chamber, removing the stopper and leaving it also in the chamber, and dry the sample at the 105 °C for 2 hours. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in a desiccator. Weigh the cool bottle and its contents (M_3).

Calculate the loss on drying from the following equation:

$$\text{Loss on drying (\%w/w)} = \frac{M_2 - M_3}{M_1} \times 100$$

where:

M_1 is the mass of sample in grams;

M_2 is the mass of sample and weighing bottle in grams before drying; and

M_3 is the mass of sample and weighing bottle in grams after drying and cooling in a desiccator.

If the sample melts at a temperature lower than 105 °C, prepare the sample as described above, then place it in a vacuum desiccator containing sulfuric acid. Evacuate the desiccator to 130 Pa (1 mm of mercury), maintain this vacuum for 24h, and then weigh the dried sample. Calculate the loss on drying using the same equation above.

Annex C

(normative)

Test for acidity and alkalinity

C. 1 Apparatus

Beaker

Measuring cylinder

C. 2 Reagents

Phenolphthalein indicator

Sodium hydroxide

C. 3 Procedure

Dissolve 1 g of sample in 10 ml of freshly boiled and cooled water. Add one drop of phenolphthalein indicator. No pink colour shall appear. Add one drop of 0.1 M sodium hydroxide. A pink colour shall appear.

C.1 Procedure

Dissolve 1 g of sample in 10 ml of freshly boiled and cooled water. Add one drop of phenolphthalein indicator. No pink colour shall appear. Add one drop of 0.1 M sodium hydroxide. A pink colour shall appear.

Annex D

(normative)

Determination of the melting range

D.1 Apparatus

D.1.1 Desiccator

D.1.2 10cm Capillary-tube

D.1.3 Standard Thermometer

D.1.4 Auxiliary Thermometer

D.2 Procedures

Dry the samples under the conditions specified for Loss on Drying under Annex B or alternatively, dry the sample for 24 h in a desiccator.

Transfer a quantity of the dried powder to a dry capillary-tube about 10 cm long and sealed at one end (thickness of the wall, 0.10-0.15 mm; i.d. 0.9-1.1 mm) and pack the powder by tapping the tube on a hard surface so as to form a tightly-packed column 2-4 mm in height.

Attach the capillary-tube and its contents to a standard thermometer so that the closed end is at the level of the middle of the bulb, and heat in a suitable apparatus containing an appropriate liquid (liquid paraffin or silicone oil) and fitted with a stirring device and an auxiliary thermometer. Regulate the rise in temperature during the first period to 3° per min. When the temperature has risen to 5° below the lowest figure of the range for the substance being tested, heat more slowly: if no other directions are given, the rate of rise in temperature should be 1-2° per min.

Unless otherwise directed, read the temperature at which the substance is observed to form droplets against the side of the tube and the temperature at which it is completely melted, as indicated by the formation of a definitive meniscus.

D.4 Calculations

To the temperature readings, apply the emergent-stem correction determined as follows: Before starting the determination of the melting range, adjust the auxiliary thermometer so that the bulb touches the standard thermometer at a point midway between the graduation for the expected melting range and the surface of the heating material. When the substance has melted, read the temperature on the auxiliary thermometer.

Calculate the correction to be added to the temperature reading of the standard thermometer from the following formula: $0.00015 N(T - t)$ in which T is the temperature reading of the standard thermometer, t is the temperature reading of the auxiliary thermometer and N is the number of degrees of the scale of the standard thermometer between the surface of the heating material and the level of the mercury.

Annex E

(normative)

Test for benzoic and salicylic acid

E.1 Procedure

Add ferric chloride dropwise to 10 ml of a hot, saturated solution of the sample. No precipitate or violet colour shall appear.

Annex F

(normative)

Test for readily carbonizable substances

F.1 Reagents

F.1.1 Sulphuric acid

F.1.2 Matching fluid composed of 0.1 ml of cobalt chloride solution (see F.1.2.1), 0.4 ml of ferric chloride solution (see E1.2.2), 0.1 ml cupric sulphate solution (see F.1.2.3) and 4.4 ml of distilled water.

F.1.2.1 Cobalt chloride solution: Dissolve 65 g of cobaltous chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) in a mixture of 25 ml of hydrochloric acid and 975 ml of distilled water. Place exactly 5 ml of this solution in a 250-ml iodine flask, add 5 ml of 3 % hydrogen peroxide solution and 15 ml of 20 % sodium hydroxide solution. Boil for 10 minutes, cool, and add 2 g of potassium iodide and 20 ml of 25 % sulphuric acid. When the precipitate has dissolved, titrate the liberated iodine with 0.1N sodium thiosulphate solution, adding starch as indicator. Each millilitre of 0.1N sodium thiosulphate is equivalent to 23.8 mg of cobaltous chloride. Adjust the final volume of the solution by adding an adequate amount of the hydrochloric acid and distilled water mixture so that each millilitre contains 59.5 mg of cobaltous chloride.

F.1.2.2 Ferric chloride solution: Dissolve 55 g of ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in a mixture of 25 ml hydrochloric acid and 975 ml of distilled water. Place 10 ml of this solution in a 250-ml iodine flask. Add 15 ml of distilled water and 3 g of potassium iodide, and allow the mixture to stand for 15 minutes. Dilute with 100 ml of distilled water, and titrate the liberated iodine with 0.1N sodium thiosulphate solution, adding starch as indicator. Each millilitre of 0.1N sodium thiosulphate is equivalent to 27.03 mg of ferric chloride. Adjust the final volume of the solution by adding enough of the hydrochloric acid and distilled water mixture to make each millilitre contain 45.0 mg of ferric chloride.

F.1.2.3 Cupric sulphate solution: Dissolve 65 g of cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in a mixture of 25 ml of hydrochloric acid and 975 ml of distilled water. Place 10 ml of this solution in a 250-ml iodine flask, add 40 ml of distilled water, 4 ml of acetic acid and 3 g of potassium iodide. Titrate the liberated iodine with 0.1N sodium thiosulphate solution, adding starch as indicator. Each millilitre of 0.1N sodium thiosulphate is equivalent to 24.97 mg of cupric sulphate. Adjust the final volume of the solution by adding enough of the hydrochloric acid and distilled water mixture to make each millilitre contain 62.4 mg of cupric sulphate.

F.2 Procedure

Dissolve 0.2 g of the sample in 5 ml of sulphuric acid. Keep the solution at 48 °C – 50 °C for 10 min. The colour of the resultant solution shall not be darker than that of the matching fluid in F.1.2 (a very light brownish-yellow).

Annex G

(normative)

Test for Selenium

G.1 Reagents

G.1.1 Selenium stock solution: Transfer 120 mg of metallic selenium (Se) into a 1 000-ml volumetric flask, add 100 ml of dilute nitric acid (1 in 2). Gently warm the solution on a steam-bath and dilute to volume with distilled water. Transfer 5 ml of this solution into a 200-ml volumetric flask, dilute to volume with distilled water, and mix. Each millilitre of this solution contains 3 µg of selenium ions (Se).

G.1.2 Standard selenium solution: Just prior to the test, transfer 20 ml of selenium stock solution (60 µg Se) into a 200 mm x 25 mm test tube, add 20 ml of hydrochloric acid, and mix.

G.1.3 Sample solution: Transfer 2 g of the sample to a 250-ml Erlenmeyer flask, and cautiously add 10 ml of 30 % hydrogen peroxide solution. After the initial reaction has subsided, add 6 ml of 70 % perchloric acid, heat slowly until white fumes of perchloric acid are copiously evolved and continue heating gently for a few minutes to ensure decomposition of any excess peroxide. If the solution is brownish in colour due to non-decomposed organic matter, add a small amount of hydrogen peroxide solution and heat again to white perchloric acid fumes, repeating, if necessary until complete decomposition of the organic matter when a colourless solution is obtained. Cool, add 10 ml of distilled water and filter into a 200 mm x 25 mm test tube. Wash the filter paper with hot water until the filtrate measures 20 ml, add 20 ml of hydrochloric acid and mix.

G.2 Procedure

Place the test tubes containing the standard selenium solution and the sample solution in a water-bath, and heat until the temperature of the solution reaches 40 °C. To each tube, add 400 mg of ascorbic acid, stir until dissolved and maintain at 40 °C for 30 min. Cool the solution, dilute with distilled water to 50 ml and mix.

Any pink colour produced by the sample shall not exceed that produced by the standard selenium solution.

Annex H

(normative)

Test for toluene sulfonamides

H.1 General

Two methods have been specified. Any method may be used depending upon the facilities available.

H.2 Method 1 – Gas Chromatography

H.2.1 Reagents

H.2.1.1 Methylene chloride: Use a suitable pure grade, equivalent to the product obtained by distillation in all glass apparatus.

H.2.1.2 Internal standard stock solution: Transfer 100 mg of 95 % n-tricosane into a 10-ml volumetric flask. Dissolve in n-heptane, dilute to volume with the same solvent, and mix.

H.2.1.3 Stock standard preparation: Transfer 20 mg each of reagent grade o-toluenesulfonamide and p-toluenesulfonamide into a 10-ml volumetric flask. Dissolve in methylene chloride, dilute to volume with the same solvent, and mix.

H.2.1.4 Diluted standard preparation: Pipette into five 10-ml volumetric flasks, 0.1 ml, 0.25 ml, 1.0 ml, 2.5 ml and 5.0 ml respectively, of the 'stock standard preparation' prepared in G.2.1.3. Pipette 0.25 ml of the 'internal standard stock solution' into each flask, dilute each to volume with methylene chloride and mix. These solutions contain 250 µg of n-tricosane, plus respectively, 20, 50, 200, 500 and 1000 µg per ml of each toluenesulfonamide, plus 250 mg of n-tricosane.

H.2.1.5 Test preparation: Dissolve 2 g of the sample in 8 ml of 5 percent sodium bicarbonate solution. Mix the solution thoroughly with 10 g of chromatographic siliceous earth (Celite 545 or equivalent). Transfer the mix into a 25 mm x 250 mm chromatographic tube having a fritted glass disk and a Teflon stopcock at the bottom, and a reservoir at the top. Pack the contents of the tube by tapping the column on a padded surface, followed by tamping firmly from the top. Place 100 ml of methylene chloride in the reservoir and adjust the stopcock so that 50 ml of eluate is collected in 20 min to 30 min. To the eluate, add 25 µl of 'internal standard stock solution'. Mix, and then concentrate the solution to a volume of 1 ml in a suitable concentrator tube fitted with a modified Snyder column, by using a Kontes tube heater maintained at 90 °C.

H.2.2 Procedure

H.2.2.1 Inject 2.5 µl of the test preparation (see G.1.1.5) into a suitable gas chromatograph equipped with a flame-ionization detector. The column should be of glass, approximately 3 m in length and 2 mm in inside diameter and packed with 3 % phenylmethyl silicone on 100 - to 120-mesh equivalent to 150- to 125-micron IS test sieve and silanised calcined diatomaceous silica.

NOTE The glass column should extend into the injector for on-column injection and into the detector base to avoid contact with metal.

The carrier is helium flowing at a rate of 30 ml per min. The injection port, column, and detector are maintained at 225 °C, 180 °C and 250 °C respectively. The instrument attenuation setting should be such that 2.5 µl of the 'diluted standard preparation' containing 200 µg per ml of each toluenesulfonamide gives a response of 40 % to 80 % of full-scale deflection. Record the chromatogram, note the peaks for o-toluenesulfonamide, p-toluenesulfonamide, and the n-tricosane internal standard, and calculate the areas for each peak by suitable means. The retention times for o-toluenesulfonamide, p-toluenesulfonamide, and n-tricosane are about 5 min, 6 min and 15 min, respectively.

DEAS 995: 2019

H.2.2.2 In a similar manner, obtain the chromatograms for 2.5- μ l portions of each of the live 'diluted standard preparations' and for each solution, determine the areas of the o-toluenesulfonamide, p-toluenesulfonamide, and n-tricosane peaks.

From the values thus obtained, prepare standard curves by plotting concentration of each toluenesulfonamide, in micrograms per millilitre versus the ratio of the respective toluenesulfonamide peak area to that of n-tricosane. From the standard curve determine the concentration, in μ g per ml, of each toluenesulfonamide in the 'test preparation'. Divide each value by 2 to convert the result to parts per million of toluenesulfonamide in the 2 g sample taken for analysis.

NOTE: If the toluenesulfonamide content of the sample is greater than 500 mg/l, the impurity may crystallize out of the methylene chloride concentrate (see G.1.1.1). Although this level of impurity exceeds that permitted by the specification, the analysis may be completed by diluting the concentrate (usually 1: 10 is satisfactory) with methylene chloride containing 250 μ g of n-tricosane per ml, and by applying appropriate dilution factors in the calculation. Care shall be taken obtain a homogeneous solution by completely dissolving any crystalline toluenesulfonamide.

H.3 Method 2 – Thin Layer Chromatography

H.3.1 Apparatus

H.3.1.1 Flat glass plates (200 mm x 100 mm)

H.3.1.2 Micropipette

H.3.1.3 Developing chamber lined with filter paper

H.3.2 Reagents

H.3.2.1 Silica gel

H.3.2.2 Chloroform

H.3.2.3 Methyl alcohol

H.3.2.4 Ammonia solution, strong

H.3.2.5 4–Sulphamoylbenzoic acid reference material

H.3.2.6 Toluene–2–sulfonamide acid reference material

H.3.2.7 Sodium hypochloride solution diluted with water to contain 0.5 % (m/v) of available chlorine

H.3.2.8 Potassium iodide

H.3.2.9 Starch mucilage

H.3.2.10 Glacial acetic acid

H.3.2.11 **Solution A:** 4 volumes of methyl alcohol plus 1 volume of acetone plus 0.5 % (m/v) of the sample

H.3.2.12 **Solution B:** 4 volumes of methyl alcohol plus 1 volume of acetone plus 0.005 % (m/v) of the 4-sulphamoylbenzoic acid

H.3.2.13 **Solution C:** 4 volumes of methyl alcohol plus 1 volume of acetone plus 0.005 % (m/v) of the toluene-2-sulfonamide.

H.3.3 Procedure

Prepare suspension of silica gel G. Spread the suspension on the plates about 0.25 mm thick. Allow to stand until the coating sets and then dry the plates at 105 °C to 110 °C for one hour. Protect the plates from moisture. Pour into the developing chamber sufficient quantity of mobile phase (100 volumes of chloroform + 50 volumes of methyl alcohol + 11.5 volume of strong ammonia solution) to form a layer about 15 mm deep. Close the tank for one hour at 20 °C to 27 °C. Remove the narrow strips of the coating, about 5 mm inside from the margins of the chromatoplate. Using micropipette apply separately to the chromatoplates 2 ml each of solutions A, B and C. These spots should be about 25 mm from the bottom of the plates and not less than 25 mm from the sides of the plates. The diameter of the spots should not be more than 6 mm. Dry the spots and place the chromatoplates in the developing chamber at 20 °C to 27 °C until the mobile phase has ascended to the 150-mm line.

Remove the plates and dry them in current of warm air. Then heat at 105 °C for five minutes. Spray the hot plates with the sodium hypochlorite solution. Dry in a current of cold air until sprayed area of the plate below the line of application give at most a faint blue colour with a drop of a mixture, prepared by dissolving 0.5 % (m/v) of potassium iodide in starch mucilage containing 1 % (m/v) of glacial acetic acid. Avoid prolonged exposure to cold air. Spray the plates with the same mixture.

The spots in the chromatograms obtained with solution (B) and (C) should be more intense than any corresponding spots in the chromatogram obtained with solution (A).

Bibliography

- [1] IS 5345: 1996 (Reaffirmed in 2001), *Sodium saccharin food grade — Specification (Second revision)*
- [2] JECFA (2001), *Saccharin* monograph
- [3] Food chemicals Codex (5th Edition)
- [4] JECFA (2006), Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications, Volume 4.