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SPECIFICATION FOR

COMPOUND FEEDS FOR DAIRY CATTLE AND BUFFALO

SRI LANKA STANDARDS INSTITUTION

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SLS 914:1991

Gr. 12

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This standard does not purport to include all the necessary provisions of a contract.

SRI LANKA STANDARD

SPECIFICATION FOR COMPOUND FEEDS FOR DAIRY CATTLE AND BUFFALO

FOREWORD

This Sri Lanka Standard was authorized for adoption and publication by the Council of the Sri Lanka Standards Institution on 1991-04-02, after the draft, finalized by the Drafting Committee on Animal Feeds, had been approved by the Agricultural and Food Products Divisional Committee.

In this specification eight types of compound feeds for dairy cattle and buffalo have been specified.

This specification is subject to the provisions of the Animal Feed Act No. 15 of 1986 and the regulations framed thereunder.

For the purpose of deciding whether a particular requirement of this specification is complied with, the final value, observed or calculated, expressing the result of a test or an analysis, shall be rounded off in accordance with CS 102. The number of significant places retained in the rounded off value shall be the same as that of the specified value in this specification.

In the preparation of this specification the assistance obtained from the Veterinary Research Institute of Sri Lanka is gratefully acknowledged.

1 SCOPE

This specification prescribes the requirements and methods of sampling and test for compound feeds for dairy cattle and buffalo.

2 REFERENCES

CS 102 Presentation of numerical values.
SLS 428 Random sampling methods.
SLS 626 Tests for animal feeds.

3 DEFINITIONS

For the purpose of this specification, the following definitions shall apply:

3.1 **compound feed** : A mixture of vegetable or animal products, or derivatives obtained therefrom, or organic or inorganic substances, whether or not containing feed additives, intended for oral feeding as a complete feed or complementary feed.

3.2 **daily ration** : The quantity of total feed required daily by an animal concerned in order to satisfy all its daily nutritional needs.

3.3 **complementary feed** : A compound feed which, by reason of its composition, is sufficient to ensure a daily ration only if fed in combination with other feeding stuffs.

3.4 **complete feed** : A compound feed which, by reason of its composition, is sufficient to ensure a daily ration.

3.5 **protein equivalent of added NPN** : 6.25 times the amount of nitrogen in the form of urea, biuret, urea phosphate or diureidoisobutane, as the case may be.

4 TYPES AND PHYSICAL FORM

4.1 Types

Compound feeds for dairy cattle and buffalo shall be of the following types:

4.1.1 *Milk replacer feed*

Complete or complementary feed intended for administration to preruminant calves, either in dry form or after reconstitution with a specified quantity of liquid, as a substitute for or supplement to post -colostral milk

4.1.2 *Calf starter feed*

Complementary feed intended for starting calves,that is calves up to about 6 months of age.

4.1.3 *Calf feed*

Complementary feed intended for growing calves, that is calves from 6 to 12 months of age.

4.1.4 *Dairy feed*

Complementary feed intended generally for all dairy cattle and buffaloes of not less than 12 months of age.

4.1.5 *Lactating cow feed*

Complementary feed intended specifically for lactating cows.

4.1.6 *Protein concentrate*

Complementary feed which contains not less than 30 per cent crude protein by mass and which is designed for mixing, before feeding, with other feeding stuffs at an inclusion rate of not less than 5 per cent by mass.

4.1.7 *Mineral supplement*

Complementary feed which is composed mainly of minerals and contains not less than 40 per cent ash by mass. It shall contain calcium, phosphorus and sodium and may contain any or all of the following :

a) Magnesium, sulfur, manganese, zinc, iron, copper, cobalt, iodine and selenium.
b) Vitamin A and its provitamins, vitamin D and vitamin E

4.1.8 *Molassed feed*

Complementary feed prepared from molasses and containing not less than 14 per cent total sugars by mass, expressed as sucrose.

4.2 Physical form

The word "feed" in calf starter feed, calf feed, dairy feed and lactating cow feed may be substituted with the word "mash", "meal" or "pellets". The word "supplement" in mineral supplement may be substituted with the word "mixture", "block" or "lick" as appropriate, in order to indicate the physical form.

5 REQUIREMENTS

5.1 Basic requirements

5.1.1 Compound feed other than mineral supplement shall comply with the basic requirements specified in Table 1, when tested by the methods prescribed in Column 10 of the table.

TABLE 1 - Basic requirements for compound feeds

Sl. No.	Characteristic	Requirement							Method of test
		Milk replacer	Calf starter	Calf feed	Dairy feed	Lactating cow feed	Protein concentrates	Molassed feed	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
i))	Moisture, per cent by mass, max.	8.0	12.0	12.0	12.0	12.0	12.0	12.0	5 of SLS 626:1983
ii)	Total protein, per cent by mass, min.	22.0	20.0	18.0	14.0	16.0	30.0	-	6 of SLS 626:1983
iii)	Oil, per cent by mass, min.	15.0	3.0	3.0	3.0	3.0	3.0	3.0	7 of SLS 626:1983
	max.	20.0	8.0	8.0	8.0	8.0	8.0	8.0	
iv)	Fibre, per cent by mass max.	1.0	7.0	10.0	12.0	12.0	10.0	12.0	8 of SLS 626:1983
v)	Lactose, per cent by mass, min.	20.0	-	-	-	-	-	-	Appendix C
vi)	Total sugars (as sucrose) per cent by mass, min.	-	-	-	-	-	-	14.0	Appendix D
vii)	calcium, per cent by mass, min.	0.6	0.6	0.5	0.5	0.8	3.0	0.5	11 of SLS 626:1983
viii)	Phosphorus, per cent by mass, min.	0.5	0.5	0.4	0.5	0.6	1.5	0.5	12 of SLS 626: 1983

NOTE

The values for characteristics ii) to viii) have been expressed on the assumption that the compound feed concerned contains 10.0 per cent moisture. Therefore, calculated values should be corrected to a 10.0 per cent moisture content.

5.1.2 Mineral supplement shall comply with the basic requirements specified in Table 2, when tested by the methods prescribed in Column 4 of the table.

TABLE 2 - **Basic requirements for mineral supplement**

Sl.No (1)	Mineral (2)	Requirement (3)	Method of test (4)
i)	Calcium, per cent by mass	5 to 25	11 of SLS 626 : 1983
ii)	Phosphorus, per cent by mass	2 to 12	12 of SLS 626 : 1883
iii)	Sodium, per cent by mass	4 to 12	Appendix E

NOTE
The values for characteristics i) to iii) have been expressed on the assumption that the feed contains 10.0 per cent moisture. Therefore, calculated values should be corrected to a 10.0 per cent moisture content.

5.2 Additional requirements

5.2.1 *Milk replacer feeds*

Milk replacer feed shall comply with the additional requirements specified in Table 3 (see Note under 5.4).

TABLE 3 - **Additional requirements for milk replacer feed**

Sl. No. (1)	Characteristic (2)	Requirement (3)
i)	Lysine, per cent by mass, min.	1.80
ii)	Methionine and cystine, per cent by mass min.	0.80
iii)	Sodium, per cent by mass, min.	0.40

NOTE

All values have been expressed on the assumption that the feed contains 10.0 per cent moisture.

5.2.2 *Mineral supplement*

Mineral supplement may contain any element specified in Column 2 of Table 4, in the range specified in Column 3 of the table (see Note under 5.4).

TABLE 4 - Additional requirements for mineral supplement

Sl. No. (1)	Characteristic (2)	Requirement (3)
i)	Magnesium, per cent by mass	0.5 to 12.5
ii)	Manganese, mg/kg	250 to 300
iii)	Zinc, mg/kg	25 to 100
iv)	Iron, mg/kg	75 to 300
v)	Copper, mg/kg	50 to 75
vi)	Cobalt, mg/kg	40 to 250
vii)	Iodine, mg/kg	50 to 100
viii)	Selenium, mg/kg	10 to 25

NOTE

All values have been expressed on the assumption that the feed contains 10.0 per cent moisture.

5.3 Undesirable Substances

Compound feeds shall not contain any substance specified in Table 5, in excess of the amounts given in Columns 3, 4 or 5 of the table.

TABLE 5 - Limits for undesirable substances

Sl. No. (1)	Substance (2)	Maximum limit, in mg/kg		
		Complete feed (3)	Complementary feed (4)	Mineral supplement (5)
i)	Arsenic	2	4	12
ii)	Cadmium	0.5	0.5	5
iii)	Fluorine	50	125	2000
iv)	Lead	5	10	30
v)	Mercury	0.1	0.2	0.2
vi)	Nitrites	15	15	-
vii)	Aflatoxin B1	0.05	0.05	-
viii)	Free gossypol	500	500	-
ix)	Total HCN	50	50	-

NOTE

All values have been expressed on the assumption that the feed contains 10.0 per cent moisture.

5.4 Use of nonprotein nitrogenous compounds

5.4.1 Compound feed intended for calves upto 6 months of age shall not contain any added nonprotein nitrogenous compound.

5.4.2 Nonprotein nitrogenous compound other than urea, biuret, urea phosphate or diureidoisobutane shall not be used as a feed additive.

NOTE
Tests for requirements given in 5.2, 5.3 and 5.4 should be carried out only if requested by the interested party.

6 PACKAGING

Compound feeds shall be packed in clean and dry bags or other suitable containers, which shall be suitably closed. Compound feeds in the form of blocks may be wrapped in paper or polythene.

7 MARKING

Each bag or container or wrapper shall be marked or labelled legibly and indelibly with the following:

a) Name of the product;
b) Type and physical form and the category of animal for which it is intended;
c) Name and address of the manufacturer (including the country of origin);
d) Trade name;
e) Date of manufacture or date of expiry;
f) Batch or code number;
g) Net mass;
h) Ingredients (see Appendix A);
j) Declarations relating to composition (see Appendix B). When nonprotein nitrogenous compounds are added, name of the compound, protein equivalent of added NPN and directions for use, indicating the level of total NPN which should not be exceeded in the daily ration; and
k) Directions for use.

NOTE
Attention is drawn to the certification facilities offered by the Sri Lanka Standards Institution. See the inside back cover of this standard.

8 SAMPLING

8.1 Lot

In any consignment all the packages of the same size and containing compound feeds of the same type and belonging to one batch of manufacture or supply shall constitute a lot.

8.2 Scale of sampling

8.2.1 Samples shall be tested from each lot for ascertaining its conformity to the requirements of this specification.

8.2.2 The number of packages to be selected from a lot shall be in accordance with Table 6.

Table 6 - Scale of sampling

No. of packages in the lot	No. of packages to be selected
Up to 50	3
51 to 100	4
101 to 250	5
251 to 500	7
501 to 1 000	10
1 001 and above	15

8.2.3 The packages shall be selected at random. In order to ensure randomness of selection tables of random numbers as given in SLS 428 shall be used.

8.3 Preparation of the sample

A sufficient quantity of material shall be taken from different parts of each package selected as in 8.2.2. The quantities so obtained from the packages shall be mixed thoroughly to form a composite sample. If reduction of composite sample is necessary then reduction shall be done by the coning and quartering method.

8.4 Number of tests

8.4.1 Each package selected as in 8.2.2 shall be inspected for packaging and marking requirements.

8.4.2 The sample prepared as in 8.3 shall be tested for the requirements given in Table 1 and Table 2.

8.4.3 A part of the sample prepared as in 8.3 shall be tested for any or all of the additional requirements given in Tables 3, 4 and 5 only if requested by the interested party.

9 METHODS OF TEST

Tests shall be carried out as prescribed in SLS 626 and Appendices C to E of this specification.

10 CRITERIA FOR CONFORMITY

A lot shall be declared as conforming to the requirements of this specification if the following conditions are satisfied

10.1 Each package inspected as in 8.4.1 satisfies the relevant requirements.

10.2 The sample tested as in 8.4.2 satisfies the relevant requirements.

APPENDIX A
PROVISIONS RELATING TO LISTING OF INGREDIENTS IN LABEL

Ingredients may be indicated by a category name as prescribed in Table 7 and Table 8.

Feed additives added purely for technological reasons may be omitted from the list of ingredients.

TABLE 7 - Straight feeds

Category	Description
Cereals	All cereals, subjected to crushing, grinding or other processing, and derivatives from their starchy endosperm.
Cereal by-products	All by-products of cereal milling.
Legumes	All legumes, subjected to crushing, grinding or other processing.
Legume by-products	All by-products of legume milling.
Leaf meal	Dried and ground leaves or leafy parts of plants.
Starchy feeds	All roots and tubers, subjected to processing, and bakery waste consisting mainly of flour.
Oilseed meals	All oilseed in meal form or residue left after extraction of oil from undecorticated or decorticated oilseeds.
Industrial by-products	All by-products of the brewery, distillery, fruit-processing, starch and sugar industries.
Milk products	All milk products and derivatives from the processing thereof.

Table 7 contd....

Category	Description
Fish products	All fish including shellfish or parts there of subjected to processing and derivatives obtained therefrom.
Animal products	All products and by-products of animal origin, other than milk and fish products.
Microbial protein	All protein sources obtained from bacteria, yeasts and algae.
Oil	All oils of animal or vegetable origin.
Fat	All fats of animal or vegetable origin.
Minerals	All inorganic substances intended as sources of minerals except trace elements in animal feeds.

TABLE 8 - **Permitted feed additives**

Category	Desctiption
Amino acids and their salts	DL -methionine, L-threonine; L-lysine, L-tryptophan, DL-tryptophan.
Amino acid salts	Dihydrated calcium salt of N-hydroxy-methyl-DL-methionine, L-lysine mono-hydrochloride, L-lysine sulphate, zinc methionine, DL-2-hydroxy-4-methylmer-capto-butyric acid and its calcium salt.
Nonprotein nitrogenous compounds	Urea, urea phosphate, diureidoisobutane, ammonium acetate, ammonium lactate.

Table 8 contd.....

Category	Description
Trace - element sources	Manganese:- Manganic oxide, manganous carbonate, manganous chloride, manganous hydrogen phosphate, manganous oxide, manganous sulfate manohydrate, manganous sulfate tetrahydrate. Zinc:-Zinc acetate, zinc carbonate, zinc chloride monohydrate, zinc lactate, zinc oxide, zinc sulfate heptahydrate, zinc sulfate monohydrate. Iron :- Ferric chloride, ferric oxide; ferrous carbonate, ferrous chloride; ferrous citrate, ferrous fumarate; ferrous lactate , ferrous sulfate. Copper :- Cupric acetate, basic cupric carbonate monohydrate, cupric chloride; cupric methionate, cupric sulfate. Cobalt :- Cobaltous acetate, basic coboltous carbonate, cobaltous chloride; cobaltous nitrate, cobaltous sulfate heptahydrate, cobaltous sulfate monohydrate. Iodine :- Anhydrous calcium iodate, calcium iodatehexahydrate, potassium iodide, sodium iodide. Molybdenum:- Ammonium molybdate, sodium molybdate. Selenium:- Sodium selenate, selenite.
Vitamins	All vitamins, provitamins and substances having a similar effect.
Anticaking agents	Sodium stearate, potassium stearate, calcium stearate, anhydrous silicic acid, kieselguhur, calcium silicate; sodium aluminosilicate, mixtures of steatite, chlorite and vermiculite and all other permitted anticaking agents.

Table 8 contd.....

Category	Description
Antioxidants	L - ascorbic acid, sodium L- ascorbate, Calcium di L-ascorbate, 5 -6 - diacetyl - L - ascorbic acid, 6 -palmitoyl - L -ascorbicacid, Alpha tocopherol, gamma tocopherol, delta tocopherol, propylgallate, octyl gallate, butylated hydroxyanisole; butylated hydroxytoluene, ethoxyquin and all other permitted antioxidants.
Emulcifiers and stabilizers	Lecithins, sodium, potassium and calcium salts of edible fatty acids, monoacyl and diacyl glycerols, monoacyl and diacyl glycerols esterified with acetic, lactic, citric, tartaric, monoacetyl tartaric and diacetyltartaric acids; sucrose esters of fatty acids; sucroglycerides, polyglycerol esters of edible fatty acids, propylene glycol esters of fatty acids, stearoyl -2- lactylic acid, sodium stearoyl - 2 -lactylate,) calcium stearoyl - 2 -lactylate, Stearyl tartrate) Glycerol poly ethylene glycol ricinoleate, sorbitan monostearate, tristearate, monolaurate, mono-oleate and monopalmitate, polyoxyethylene sorbitan monolaurate, mono-oleate, monopalmitate, monostearate, tristearate and trioleate; polyoxyethylated glycerides of fatty acids; polyethyleneglycol esters of fatty acids, and all other permitted emulcifiers and stabilizers.
Preservatives	Sorbic acid, sodium sorbate, potassium and calcium sorbate, formic acid; ammonium, sodium and calcium formate; acetic acid, sodium, potassium and calcium acetate, Lactic acid, Sodium, potassium and calcium lactate; Propionic acid, ammonium, calcium, sodium and potassium propionate; DL -malic acid, fumaric acid, citric acid; sodium, potassium and calcium citrate; L -tartaric acid,sodium and potassium L-tartrates, orthophosphoric acid and all other permitted preservatives.
Mould inhibitor	Any permitted mould inhibitor.

APPENDIX B
DECLARATION OF THE COMPOSITION

B.1 COMPULSORY DECLARATIONS

B.1.1 Compound feeds other than molassed feed or mineral supplement, shall be marked or labelled with the following:

a) Total protein, per cent by mass;
b) Oil, per cent by mass;
c) Fibre, per cent by mass; and
d) Ash, per cent by mass.

B.1.2 Molassed feed shall be marked or labelled with the following:

a) Total sugars, per cent by mass;
b) Protein, per cent by mass;
c) Fibre, per cent by mass; and
d) Ash, per cent by mass.

B.1.3 Mineral supplement shall be marked or labelled with the following:

a) Calcium, per cent by mass;
b) Phosphorus, per cent by mass;
c) Sodium, per cent by mass;
d) Magnesium, if present in excess of 0.5 per cent by mass;
e) Copper, mg/kg, if present; and
f) Vitamin A, D or E, mg/kg, if present.

B.2 OPTIONAL DECLARATIONS

B.2.1 Compound feeds other than milk replacer feed or mineral supplement, shall be marked or labelled with the following:

a) Moisture, per cent by mass;
b) Calcium, per cent by mass;
c) Phosphorus, per cent by mass;
d) Sodium, per cent by mass; and
e) Magnesium, per cent by mass.

B.2.2 Milk replacer feed shall be marked or labelled with the following:

a) Moisture, per cent by mass;
b) Lysine, per cent by mass;
c) Methionine and cystine, per cent by mass;
d) Calcium, per cent by mass;
e) Phosphorus, per cent by mass;
f) Sodium, per cent by mass; and
g) Magnesium, per cent by mass.

B.2.3 Mineral supplement shall be marked or labelled with the following:

a) Trace elements other than copper, mg/kg;
b) Protein, per cent by mass;
c) Oil, per cent by mass;
d) Fibre, per cent by mass; and
e) Ash, per cent by mass.

APPENDIX C
DETERMINATION OF LACTOSE

C.1 REAGENTS

C.1.1 *Saccharomyces cerevisiae suspension*

Suspend 25 g of the fresh yeast in 100 ml of water. Store in a refrigerator and use within one week.

C.1.2 *Carrez solution I*

Dissolve 21.9 g of zinc acetate dihydrate in water. Add 3 ml of glacial acetic acid and dilute to 100 ml.

C.1.3 *Carrez solution II*

Dissolve 10.6 g of potassium ferrocyanide in water and dilute to 100 ml.

C.1.4 *Luff-Schoorl reagent*

C.1.4.1 *Citric acid solution*

Dissolve 50 g of citric acid monohydrate in water and dilute to 50 ml.

C.1.4.2 *Copper sulfate solution*

Dissolve 25 g of copper sulfate pentahydrate in water and dilute to 100 ml.

C.1.4.3 *Sodium carbonate solution*

Dissolve 143.8 g of anhydrous sodium carbonate in about 300 ml of warm water in a 1000-ml volumetric flask and allow to cool.

C.1.4.4 *Reagent preparation*

Add citric acid solution (C.1.4.1) and copper sulfate solution (C.1.4.2) while swirling, to the sodium carbonate solution (C.1.4.3) and dilute to 1000 ml with water. Leave to settle overnight and filter.

C.1.5 *Potassium iodide,* 20 g in 100 ml of water.

C.1.6 *Sulfuric acid,* 3 mol/l solution.

C.1.7 *Sodium thiosulfate,* standard volumetric soltuion, $c(Na_2S_2O_3) = 0.05$ mol/l.

C.1.8 *Starch indicator solution,* dissolve 5 g of starch in 30 ml of water. Add it to 1000 ml of boiling water and boil for 3 minutes. Allow to cool and add 10 mg of mercuric iodide as a preservative.

C.2 APPARATUS

C.2.1 *Volumetric flask,* of 100-ml capacity.

C.2.2 *Water bath*

C.2.3 *Conical flask,* of 300-ml capacity with a ground glass neck and a reflux condenser.

C.3 PROCEDURE

Weigh, to the nearest milligram, about 1 g of the sample. Transfer to the volumetric flask (C.2.1) and add 25 ml to 30 ml of water. Place in a boiling water bath for 30 minutes and cool to about 35 °C. Add 5 ml (see Note) of yeast suspension (C.1.1). Mix and place in a water bath maintained at 39 ± 1°C for 2 hours. Cool to about 20°C. Add 2.5 ml of Carrez solution I (C.1.2) and stir for 30 seconds. Add 2.5 ml of Carrez solution II (C.1.3) and stir again for 30 seconds. Make up to the volume with water and filter. Transfer a suitable volume of the filtrate containing 40 mg to 50 mg of lactose to the conical flask (C.2.3). Make up to 25 ml with water if necessary. Add few granules of pumice stones and 25 ml of Luff-Schoorl reagent (C.1.4). Heat the flask while swirling and bring to boil in about 2 minutes. Fix the reflux condenser and boil for exactly 10 minutes. Cool the flask immediately.

After about 5 minutes add 10 ml of potassium iodide solution (C.1.5) and immediately add 25 ml of sulfuric acid (C.1.6) in small quantities to prevent violent foaming. Titrate with sodium thiosulfate solution (C.1.7) to dull yellow colour. Add starch indicator solution (C.1.8) and complete the titration.

NOTE

If the sample contains more than 40 per cent fermentable sugar, use a large volume of yeast suspension as appropriate.

Carry out a blank titration by omitting the sample. Mix 25 ml of water, 25 ml of luff-Schoorl reagent (C.1.4), 10 ml of potassium iodide solution (C.1.5) and 25 ml of sulfuric acid (C.1.6). Titrate as before without boiling.

C.4 RESULTS

Calculate the difference of the volume in the sample titration and blank titration. From Table 9 determine the lactose content equivalent to corrected volume. Express as a percentage.

TABLE 9 - Values for 25 ml of Luff-Schoorl reagent

Volume of 0.05 mol/l sodium thiosulfate (ml)	Lactose content (mg)
1	3.6
2	7.3
3	11.0
4	14.7
5	18.4
6	22.1
7	25.8
8	29.5
9	33.2
10	37.0
11	40.8
12	44.6
13	48.4
14	52.2
15	56.0
16	59.9
17	63.8
18	67.7
19	71.7
20	75.7
21	79.8
22	83.9
23	88.0

APPENDIX D
DETERMINATION OF TOTAL SUGARS

D.1 REAGENTS

D.1.1 *Neutral ethyl alcohol*, 40 per cent (V/V) solution.

D.1.2 *Carrez solution I*, prepared as in **C.1.2.**

D.1.3 *Carrez solution II*, prepared as in **C.1.3.**

D.1.4 *Ethyl alcohol*, 80 per cent (V/V) solution.

D.1.5 *Methyl orange*

D.1.6 *Hydrochloric acid*, 4 mol/l solution.

D.1.7 *Hydrochloric acid*, 0.1 mol/l solution.

D.1.8 *Sodium hydroxide*, 0.1 mol/l solution.

D.1.9 *Luff-Schoorl reagent*, prepared as in **C.1.4.**

D.1.10 *3-Methylbutan - 1 - ol*

D.1.11 *Potassium iodide*, 30 g in 100 ml of water.

D.1.12 *Sulfuric acid*, 3 mol/l solution

D.1.13 *Sodium thiosulfate*, standard volumetric solution, $c(Na_2S_2O_3) = 0.05$ mol/l.

D.1.14 *Starch indicator solution*, prepared as in **C.1.8.**

D.2 APPARATUS

D.2.1 *Volumetric flask*, of 1000-ml capacity.

D.2.2 *Rotary shaker*, having a speed of 35 rpm to 40 rpm.

D.2.3 *Rotary evaporator*

D.2.4 *Conical flask*, of 300-ml capacity with a ground-glass neck and a reflux condenser.

D.3 PROCEDURE

D.3.1 *Extraction of sugar from the sample*

Weigh, to the nearest milligram, about 20 g of the sample. Place in a 1000-ml volumetric flask (D.2.1) and add 500 ml of water. Shake for one hour in the rotary shaker (D.2.2). Add 20 ml of Carrez solution 1 (D.1.2) and shake for 1 minute. Add 20 ml of Carrez solution 11 (D.1.3) and shake for 1 minute. Dilute to 1000 ml with ethyl alcohol (D.1.4) and filter. Take 200 ml aliquot of the filtrate and evaporate to about half the volume in the rotary evaporator. Transfer the residue to a 200-ml volumetric flask with warm water. Allow to cool and dilute to the mark with water. Filter if necessary.

D.3.2 *Inversion of sugars*

Place a 50 ml aliquot of ethanol-free extract (D.3.1) in a 100-ml volumetric flask. Add a few drops of methyl orange indicator. While swirling add hydrochloric acid (D.1.6) until colour turns red. Add 15 ml of hydrochloric acid (D.1.7) and immerse the flask in a water bath maintained at 100 $\pm$ 2°C for 30 minutes. Cool rapidly to 20 °C. Add 15 ml of sodium hydroxide (D.1.8) and dilute to the volume with water.

D.3.3 *Titration*

Add 25 ml of Luff-Schoorl reagent (D.1.9) and few grannules of pumice stones to the conical flask (D.2.4). Add 25 ml of the inverted sugar (D.3.2) and 1 ml of 3 - methylbutan - 1- ol. Bring to boil within 2 minutes while swirling manually. Fix the reflux condenser and boil the flask for exactly 10 minutes. Cool immediately in cold water. After 5 minutes add 10 ml of potassium iodide solution (D.1.11) and immediately add 25 ml of sulfuric acid (D.1.12) in small quantities to prevent violent foaming. Titrate with sodium thiosulfate (D.1.13) to dull yellow colour. Add starch indicator (D.1.14) and complete the titration.

Carryout a blank titration using 25 ml of water instead of 25 ml of inverted sugar extract.

D.4 RESULTS

Calculate the difference of the volume in the sample titration and the blank titration. From Table 10 determine the glucose content equivalent to this volume. Multiply it by 0.95 to give the total sugars as sucrose. Express as a percentage.

TABLE 10 - Values for 25 ml of Luff-Schoorl reagent

Volume of 0.05 mol/l sodium thiosulfate (ml)	Glucose content (mg)
1	2.4
2	4.8
3	7.2
4	9.7
5	12.2
6	14.7
7	17.2
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3
17	44.2
18	47.1
19	50.0
20	53.0
21	56.0
22	59.1
23	62.2

APPENDIX E
DETERMINATION OF SODIUM

E.1 REAGENTS

E.1.1 *Dilute hydrochloric acid*

Mix two volumes of hydrochloric acid (rel.den. = 1.18) with one volume of water.

E.1.2 *Releasing agent*

Dissolve 50 g of caesium chloride and 250 g of aluminium nitrate in water and dilute to 1000 ml. Store in a plastic bottle.

E.1.3 *Standard sodium solution*

Dissolve 2.542 g of sodium chloride in water. Add 5 ml of dilute hydrochloric acid (E.1.1). Dilute to 1000 ml and store in a plastic bottle.

1 ml of this solution is equivalent to 1 mg of sodium.

E.2 APPARATUS

E.2.1 *Crucible with a lid*, of platinum, silica or porcelain.

E.2.2 *Muffle furnace*, maintained at 450 $\pm$ 10°C.

E.2.3 *Water bath*, maintained at 90 $\pm$ 2°C.

E.2.4 *Flame photometer*

E.3 PROCEDURE

E.3.1 *Preparation of the calibration curve*

Transfer 10 ml of the standard solution (E.1.3) to a 250-ml volumetric flask. Dilute to the volume with water and mix.

Transfer into a series of 100-ml volumetric flasks 0 ml, 5 ml, 10 ml, 15 ml, 20 ml and 25 ml of the diluted standard solution. To each flask, add 10 ml of the releasing agent (E.1.2). Make up to the mark with water and mix.

Measure the sodium emission in the flame photometer at a wave length of 589 nm. Plot the emission against the concentration.

E.3.2 *Determination*

Weigh, to the nearest milligram, about 10 g of the sample in the crucible (**E.2.1**). Ash the material (see Note) at 450 ± 10°C for 3 hours. Transfer the ash to a 500-ml flask using about 250 ml of water and then 50 ml of dilute hydrochloric acid (**E.1.1**). Place the flask in a water bath maintained at 90 ± 2°C for 2 hours. Stir occasionally. Allow to cool. Dilute to the volume with water and filter. Discard the first 25 ml of the filtrate. Transfer an aliquot containing not more than 1 mg of sodium to 100-ml volumetric flask. Dilute the filtrate if necessary. Add 10 ml of the releasing agent (**E.1.2**) and dilute to the volume with water and mix.

NOTE
If no organic matter is present, dissolve the sample directly without ashing.

Measure the sodium emission in the flame photometer at a wave length of 589 nm. Determine the concentration of sodium by referring to the calibration curve (E.3.1). Express as a percentage.

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