



DRAFT EAST AFRICAN STANDARD

Carbonated and non-carbonated soft drinks — Specification

DRAFT FOR PUBLIC REVIEW

EAST AFRICAN COMMUNITY

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Foreword

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

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. 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 procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that “Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose”.

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.

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Introduction

Carbonated and non-carbonated soft drinks have a significant economic input in the economies of East Africa. Various brands of these beverages are produced and consumed in this country throughout the year, but more in the hot periods than in the cooler periods.

The quality of a carbonated or non-carbonated beverage depends on the quality of the various ingredients that go in its manufacture, water, acidulants, sweetening agents, emulsifiers, stabilizers, flavour, colour and carbon dioxide being the most important ones.

In view of the different varieties of carbonated and non-carbonated beverages produced in the country, it has not been possible to include in the standard the exact, or even the range of, proportions of different ingredients required for the different varieties of the beverages. This First Revision of the standard has incorporated the limits for permitted non-nutritive sweeteners, microbiological organisms, and heavy metal contaminants.

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Carbonated and non-carbonated soft drinks — Specification.

1 Scope

This draft East African Standard specifies the requirements, sampling, and test methods of test for carbonated and non-carbonated soft drinks which may be concentrated (solid or liquid) or ready to drink.

This standard does not apply to products for which other standards apply such as:

- a) waters (including packaged water, flavoured drinking water and packaged natural mineral waters);
- b) fruit drinks.
- c) fruit juices, pulp, puree, and nectars.
- d) vegetable juices and nectars.
- e) herbal juices (ready to drink and concentrates); and
- f) cereal based beverages.

2 Normative references

AOAC Official Method 999.10 Lead, Cadmium, Zinc, Copper, and Iron in Foods Atomic Absorption Spectrophotometry after Microwave Digestion First Action 1999 NMLK–AOAC Method

CAC/GL 50 General Guidelines on Sampling

CXG 66 Guidelines for the Use of Flavourings

CXS 192, General standard for food additives

EAS 12, Potable water — Specification

EAS 149, Carbon dioxide for beverage industry — Specification

EAS 35, Fortified edible salt - Specification.

EAS 38, Labelling of pre-packaged foods — General requirements.

EAS 39, Code of practice for hygiene in the in the food and drink manufacturing industry

EAS 803, Nutrition labelling — Requirements

EAS 804, Claims on foods — Requirements.

EAS 805, Use of nutrition and health claims — Requirements

ISO 2447:1998, Fruit and vegetable products — Determination of tin content

ISO 6634:1982, Fruits, vegetables, and derived products — Determination of arsenic content — Silver diethyldithiocarbamate spectrophotometric method

ISO 1842, Fruit, and vegetable products — Determination of pH

ISO 6633, Fruits, vegetables, and derived products — Determination of lead content — Flameless atomic absorption spectrometric method

ISO 4833-1, Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique

ISO 4833-2, Microbiology of the food chain — Horizontal method for the enumeration of microorganisms —

Part 2: Colony count at 30 °C by the surface plating technique

ISO 4832, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique

ISO 21527-2, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0.95

ISO 2173, Fruit, and vegetable products — Determination of soluble solids — Refractometric method

ISO 2448, Fruit, and vegetable products — Determination of ethanol content

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/ob>

3.1

Carbonated and non-carbonated soft drinks

Non-alcoholic water- based beverages with or without dissolved carbon dioxide, packed in hermetically sealed containers.

3.2

one gas (carbonation) volume

amount of carbon dioxide the water volume absorbs at the standard atmospheric pressure at 15.560 °C.

3.3

carbonation

The addition of carbon dioxide into beverages through dissolving the gas in potable water/soft drinks under controlled conditions of temperature and pressure to get the required gas volume.

3.4

[energy drink]

[soft drink that typically contains high levels of nutrients and other ingredients such as caffeine, taurine, and carnitine and are marketed as boosting energy.]

3.5

hermetically sealed containers

containers which are designed and intended to protect the contents against the entry of viable microorganisms after closing.

3.6

food grade packaging

packaging which will safeguard the hygienic, nutritional, technological and organoleptic qualities of the product and shall not impart any toxic substance or undesirable odour or flavour to the product.

4 Product description

4.2.1 Carbonated soft drinks:

Includes water based flavoured drinks with added carbon dioxide with nutritive, non-nutritive and/or intense sweeteners and other permitted food additives. Soft drinks should include sodas (soft drinks with added carbon dioxide, sweetener, and flavour) such as colas, pepper-types, root beer, lemon-lime, citrus types, diet/light/lite and regular types. These beverages may be clear, cloudy, or may contain particulated matter (for example, fruit pieces). [Includes so-called “energy” drinks that are carbonated and contain high levels of nutrients and other ingredients (for example, caffeine, taurine, and carnitine).]

4.2.2 non-carbonated soft drinks,

Includes punches and ades: Include water based flavoured drinks without added carbon dioxide, with or without addition of fruit and vegetable juice-based drinks (for example, almond, aniseed, coconut-based drinks, and ginseng drink), fruit flavoured ades (for example, lemonade, orangeade), [squashes (citrus-based soft drinks)], capile groselha, lactic acid beverage, ready-to-drink coffee and tea drinks with or without milk or milk solids, and herbal-based drinks (e.g. iced tea, fruit-flavoured iced tea, chilled canned cappuccino drinks) and “sports” drinks containing electrolytes. These beverages may be clear or contain particulated matter (for example, fruit pieces), and may be unsweetened or sweetened with sugar or a non-nutritive high-intensity sweetener. Includes so-called [“energy” drinks that are non-carbonated and contain high levels of nutrients and other ingredients (for example, caffeine, taurine, and carnitine).]

4.2.3 Concentrates (liquid or solid) for carbonated and non-carbonated soft drinks:

Include powder, syrup, liquid, and frozen concentrates for the preparation of carbonated or non-carbonated water-based non-alcoholic beverages by addition of water or carbonated water. Examples include fountain syrups (e.g., cola syrup), fruit syrups, frozen or powdered concentrate for lemonade and iced tea mixes.

5 Requirements

5.1 Composition requirements

5.1.1 Carbonated and non-carbonated soft drinks may be prepared with the following ingredients, complying with relevant standards. They include but not limited:

5.1.1.1 Water complying with the requirements of EAS 12.

5.1.1.2 Carbon dioxide complying with the requirements of EAS 149.

5.1.1.3 Sweetening agents

Carbonated and non-carbonated soft drinks may contain one or more nutritive and /or non-nutritive sweeteners, in accordance with the stipulated relevant standards.

5.1.1.4 edible common salt.

Edible salt if used in the manufacture of carbonated and non-carbonated soft drinks shall comply with the requirements of EAS 35.

5.1.1.3 Fortification

For the purposes of product fortification, essential nutrients such as vitamins and minerals may be added to carbonated and non-carbonated soft drinks.

5.1.1.4 fruit juice, fruit pulp, fruit puree, vegetable extracts, herbal extracts or extracts from other plant parts complying with relevant standards.

5.1.1.5 Flavouring agents in accordance with CXG 66

5.1.1.6 Quinine salts, if used shall comply with requirements given in table 1.

5.2 General requirements

5.2.1 Carbonated and non-carbonated soft drinks shall:

- a) have a uniform appearance and consistency.
- b) be free from off-flavours and odours.
- c) have not undergone any kind of deterioration or spoilage.
- d) be free from insect and rodent contamination.
- e) be clean and free from foreign and extraneous matter.
- f) Clear carbonated soft drinks shall be of sparkling clarity and shall remain so when stored under normal storage conditions.
- g) Clear non-carbonated soft drinks shall remain so when stored under normal storage conditions.
- h) show no sedimentation except in particulate drinks that contain particles such as pieces of fruits or vegetables.

4 Specific requirements

Carbonated soft drinks shall comply with the specific requirements in Table 1 when tested in accordance with the methods specified therein.

Table 1 — Specific requirements for carbonated and non- carbonated soft drinks.

S/N	Characteristic	Requirement	Test method
i.	pH	2.5 to 4.5	ISO 1842
ii.	Ethyl alcohol content, %vol/vol, Max	0.5	ISO 2448
iii.	Caffeine content, mg/l, Max	200	Annex B
iv.	Quinine salts, mg/l, max	100	Annex D
v.	Total soluble solids, %, min.	5	ISO 2173
vi.	Carbon dioxide, CO ₂ , min. (for carbonated soft drinks)	^a One gas volume	Annex A

[Note 1 When caffeine is used as a functional ingredient such as in energy drinks or caffeinated drinks, the amount of caffeine in the drink as consumed shall not exceed 320 mg/L when determined in accordance with Annex B.]

Note 2 The total soluble solids do not apply to carbonated water, energy reduced and zero sugar carbonated and non-carbonated drinks.

^a 1 volume of carbonation equals to 1.97667 g/l

6 Food additives

The use of food additives shall be in accordance with CXS192.

7 Contaminants

7.1 Pesticide residues

Carbonated and non-carbonated soft drinks shall comply with the pesticide residue limits prescribed by the Codex Alimentarius Commission of the respective commodity.

7.2 Heavy metal

Carbonated and non-carbonated soft drinks shall not contain heavy metal contaminants more than the limits specified in Table 2 when tested in accordance with the methods specified therein.

Table 2 — Heavy metal contaminants limits for Carbonated and non-carbonated soft drinks.

S/N	Heavy metal	Maximum limit, mg/kg	Method of test
i.	Arsenic (As), mg/kg	0.1	ISO 6634
ii.	Lead (Pb), mg/kg	0.1	ISO 6633
iii.	Tin (Sn), mg/kg (for canned products)	150	ISO 2447
iv.	Cadmium (Cd)	0.003	AOAC 999.10

8 Hygiene

8.1 Carbonated and non-carbonated soft drinks shall be produced and handled under hygienic conditions in accordance with EAS 39.

8.2 Carbonated and non-carbonated soft drinks shall comply with the microbiological limits given in Table 3 when tested in accordance with the methods specified therein.

Table 3 — Microbiological limits for carbonated and non-carbonated soft drinks.

S/N	Microorganism	limit	Method of test
i.	Total plate count, cfu/per ml, max	25	ISO 4833-1& 2
ii.	Coliform count, cfu/100 ml, max	Absent	ISO 4832
iii.	Yeast and Mould Count, cfu/ml, max	10	ISO 21527- 1 & 2

9 Packaging

9.1 Carbonated and non-carbonated soft drinks shall be packaged in food grade packaging that will safeguard the hygienic, nutritional, technological, and organoleptic qualities of the product.

10 Labelling

In addition to the requirements of EAS 38, EAS 803, EAS 804 and EAS 805, the following specific labelling requirements shall apply and shall be legibly and indelibly marked on each container, cap or label.

- a) Name of product.
- b) Name and address of manufacturer.
- c) Net volume of contents in ml, cl or l.
- d) Date of manufacture in code or in clear.
- e) Expiry

11 Sampling

Sampling of carbonated and non-carbonated soft drinks shall be done in accordance with CAC/GL 50.

[7 Sampling of carbonated beverages]

7.1 Scale of Sampling

7.1.1 Lot

All bottles in a consignment belonging to the same batch of manufacture shall constitute a lot. If the consignment is declared to consist of different batches of manufacture, bottles of the same batch shall be grouped together, and each group so formed shall constitute a separate lot.

Sample shall be tested from each lot for ascertaining conformity to the requirements of the standard.

7.1.2 The number of bottles to be selected from a lot for testing for microbiological and other requirements shall depend on the size of the lot and shall be in accordance with Table 2.

Table 2 — Number of bottles to be selected for sampling.

No. of bottles in the lot	No. of bottles to be selected	
	Microbiological	Other tests
up to 1300	12	18
1301 to 3200	18	24
3201 and above	24	30

7.1.3 The bottle to be selected for testing shall be chosen at random from the lot by the following procedure. Starting from any bottle, count them as 1, 2, 3... up to r . Every r th bottle thus counted shall be withdrawn r being the integral part of N/n , where N is the total number of bottles in the lot and n is the total number of bottle to be chosen.

7.2 Test samples and reference samples

7.2.1 Samples for microbiological tests

The sample bottle selected for microbiological tests (see col. 2 of Table 2) shall be divided at random into three equal sets and labeled with all particulars of sampling. One of these sets of sample bottles shall be for the purchaser; another for the vendor and the third set is the reference.

7.2.2 Samples for other tests

The sample bottles selected for other tests (see col. 3 of Table 2) shall be divided at random into three equal sets and labeled with all the particulars of sampling. One of these sets of sample bottles shall be for the purchaser, another for the vendor and third is the reference.

7.2.3 Referee samples

Referee samples shall consist of set of sample bottles for microbiological tests (see A.2.1) and a set of sample bottles for other tests (see A.2.2) and shall bear the seals of the purchaser agreed to between the two.

Annex A

(informative)

Method of measuring gas volume

A.1 Principle

The apparatus consists of pressure gauge having a hollow spike with holes in its side. The bottle is inserted from the side into the slot provided in the neck of the carbon dioxide tester and is secured in place by tightening with a threaded system. The pressure gauge is inserted until the needlepoint touches the crown cork. There is a shift value on the gauge stem, which is kept closed until the needlepoint of the pressure gauge is forced through the crown cork. The reading is noted on the gauge.

A.2 Procedure

Clamp the bottle in the frame of the gas volume tester. Pierce the crown cork but do not shake the bottle. Sniff off the top gas quickly until the gauge reading drops to zero. Make certain to close the valve the instant the needle touches zero in the pressure gauge. Shake the bottle vigorously until the gauge gives the reading that additional shaking does not change. Record the pressure. Note the temperature and record. Obtain the volume of gas from Table 3.

Table 4 — Carbon dioxide chart-volumes of carbon dioxide gas dissolved by one volume of water (Clause A.2.1)

Cavities Pressure in kg/cm ²		Temperature °C		0		0.14		0.28		0.42		0.56		0.70		0.84		0.98		1.12		1.27		1.41		1.55		1.69		1.83		1.97		2.11		2.25		2.39		2.53		2.67		2.81		2.95		3.09		3.23		3.37		3.52		3.66																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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0.6	1.71	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1	5.3	5.5	5.7	5.9	6.1	6.3	6.5	6.7	6.9	7.1	7.3	7.5	7.7	7.9	8.1	8.3	8.5	8.7	8.9	9.1	9.3	9.5	9.7	9.9	10.1	10.3	10.5	10.7	10.9	11.1	11.3	11.5	11.7	11.9	12.1	12.3	12.5	12.7	12.9	13.1	13.3	13.5	13.7	13.9	14.1	14.3	14.5	14.7	14.9	15.1	15.3	15.5	15.7	15.9	16.1	16.3	16.5	16.7	16.9	17.1	17.3	17.5	17.7	17.9	18.1	18.3	18.5	18.7	18.9	19.1	19.3	19.5	19.7	19.9	20.1	20.3	20.5	20.7	20.9	21.1	21.3	21.5	21.7	21.9	22.1	22.3	22.5	22.7	22.9	23.1	23.3	23.5	23.7	23.9	24.1	24.3	24.5	24.7	24.9	25.1	25.3	25.5	25.7	25.9	26.1	26.3	26.5	26.7	26.9	27.1	27.3	27.5	27.7	27.9	28.1	28.3	28.5	28.7	28.9	29.1	29.3	29.5	29.7	29.9	30.1	30.3	30.5	30.7	30.9	31.1	31.3	31.5	31.7	31.9	32.1	32.3	32.5	32.7	32.9	33.1	33.3	33.5	33.7	33.9	34.1	34.3	34.5	34.7	34.9	35.1	35.3	35.5	35.7	35.9	36.1	36.3	36.5	36.7	36.9	37.1	37.3	37.5	37.7	37.9	38.1	38.3	38.5	38.7	38.9	39.1	39.3	39.5	39.7	39.9	40.1	40.3	40.5	40.7	40.9	41.1	41.3	41.5	41.7	41.9	42.1	42.3	42.5	42.7	42.9	43.1	43.3	43.5	43.7	43.9	44.1	44.3	44.5	44.7	44.9	45.1	45.3	45.5	45.7	45.9	46.1	46.3	46.5	46.7	46.9	47.1	47.3	47.5	47.7	47.9	48.1	48.3	48.5	48.7	48.9	49.1	49.3	49.5	49.7	49.9	50.1	50.3	50.5	50.7	50.9	51.1	51.3	51.5	51.7	51.9	52.1	52.3	52.5	52.7	52.9	53.1	53.3	53.5	53.7	53.9	54.1	54.3	54.5	54.7	54.9	55.1	55.3	55.5	55.7	55.9	56.1	56.3	56.5	56.7	56.9	57.1	57.3	57.5	57.7	57.9	58.1	58.3	58.5	58.7	58.9	59.1	59.3	59.5	59.7	59.9	60.1	60.3	60.5	60.7	60.9	61.1	61.3	61.5	61.7	61.9	62.1	62.3	62.5	62.7	62.9	63.1	63.3	63.5	63.7	63.9	64.1	64.3	64.5	64.7	64.9	65.1	65.3	65.5	65.7	65.9	66.1	66.3	66.5	66.7	66.9	67.1	67.3	67.5	67.7	67.9	68.1	68.3	68.5	68.7	68.9	69.1	69.3	69.5	69.7	69.9	70.1	70.3	70.5	70.7	70.9	71.1	71.3	71.5	71.7	71.9	72.1	72.3	72.5	72.7	72.9	73.1	73.3	73.5	73.7	73.9	74.1	74.3	74.5	74.7	74.9	75.1	75.3	75.5	75.7	75.9	76.1	76.3	76.5	76.7	76.9	77.1	77.3	77.5	77.7	77.9	78.1	78.3	78.5	78.7	78.9	79.1	79.3	79.5	79.7	79.9	80.1	80.3	80.5	80.7	80.9	81.1	81.3	81.5	81.7	81.9	82.1	82.3	82.5	82.7	82.9	83.1	83.3	83.5	83.7	83.9	84.1	84.3	84.5	84.7	84.9	85.1	85.3	85.5	85.7	85.9	86.1	86.3	86.5	86.7	86.9	87.1	87.3	87.5	87.7	87.9	88.1	88.3	88.5	88.7	88.9	89.1	89.3	89.5	89.7	89.9	90.1	90.3	90.5	90.7	90.9	91.1	91.3	91.5	91.7	91.9	92.1	92.3	92.5	92.7	92.9	93.1	93.3	93.5	93.7	93.9	94.1	94.3	94.5	94.7	94.9	95.1	95.3	95.5	95.7	95.9	96.1	96.3	96.5	96.7	96.9	97.1	97.3	97.5	97.7	97.9	98.1	98.3	98.5	98.7	98.9	99.1	99.3	99.5	99.7	99.9	100.1	100.3	100.5	100.7	100.9	101.1	101.3	101.5	101.7	101.9	102.1	102.3	102.5	102.7	102.9	103.1	103.3	103.5	103.7	103.9	104.1	104.3	104.5	104.7	104.9	105.1	105.3	105.5	105.7	105.9	106.1	106.3	106.5	106.7	106.9	107.1	107.3	107.5	107.7	107.9	108.1	108.3	108.5	108.7	108.9	109.1	109.3	109.5	109.7	109.9	110.1	110.3	110.5	110.7	110.9	111.1	111.3	111.5	111.7	111.9	112.1	112.3	112.5	112.7	112.9	113.1	113.3	113.5	113.7	113.9	114.1	114.3	114.5	114.7	114.9	115.1	115.3	115.5	115.7	115.9	116.1	116.3	116.5	116.7	116.9	117.1	117.3	117.5	117.7	117.9	118.1	118.3	118.5	118.7	118.9	119.1	119.3	119.5	119.7	119.9	120.1	120.3	120.5	120.7	120.9	121.1	121.3	121.5	121.7	121.9	122.1	122.3	122.5	122.7	122.9	123.1	123.3	123.5	123.7	123.9	124.1	124.3	124.5	124.7	124.9	125.1	125.3	125.5	125.7	125.9	126.1	126.3	126.5	126.7	126.9	127.1	127.3	127.5	127.7	127.9	128.1	128.3	128.5	128.7	128.9	129.1	129.3	129.5	129.7	129.9	130.1	130.3	130.5	130.7	130.9	131.1	131.3	131.5	131.7	131.9	132.1	132.3	132.5	132.7	132.9	133.1	133.3	133.5	133.7	133.9	134.1	134.3	134.5	134.7	134.9	135.1	135.3	135.5	135.7	135.9	136.1	136.3	136.5	136.7	136.9	137.1	137.3	137.5	137.7	137.9	138.1	138.3	138.5	138.7	138.9	139.1	139.3	139.5	139.7	139.9	140.1	140.3	140.5	140.7	140.9	141.1	141.3	141.5	141.7	141.9	142.1	142.3	142.5	142.7	142.9	143.1	143.3	143.5	143.7	143.9	144.1	144.3	144.5	144.7	144.9	145.1	145.3	145.5	145.7	145.9	146.1	146.3	146.5	146.7	146.9	147.1	147.3	147.5	147.7	147.9	148.1	148.3	148.5	148.7	148.9	149.1	149.3	149.5	149.7	149.9	150.1	150.3	150.5	150.7	150.9	151.1	151.3	151.5	151.7	151.9	152.1	152.3	152.5	152.7	152.9	153.1	153.3	153.5	153.7	153.9	154.1	154.3	154.5	154.7	154.9	155.1	155.3	155.5	155.7	155.9	156.1	156.3	156.5	156.7	156.9	157.1	157.3	157.5	157.7	157.9	158.1	158.3	158.5	158.7	158.9	159.1	159.3	159.5	159.7	159.9	160.1	160.3	160.5	160.7	160.9	161.1	161.3	161.5	161.7	161.9	162.1	162.3	162.5	162.7	162.9	163.1	163.3	163.5	163.7	163.9	164.1	164.3	164.5	164.7	164.9	165.1	165.3	165.5	165.7	165.9	166.1	166.3	166.5	166.7	166.9	167.1	167.3	167.5	167.7	167.9	168.1	168.3	168.5	168.7	168.9	169.1	169.3	169.5	169.7	169.9	170.1	170.3	170.5	170.7	170.9	171.1	171.3	171.5	171.7	171.9	172.1	172.3	172.5	172.7	172.9	173.1	173.3	173.5	173.7	173.9	174.1	174.3	174.5	174.7	174.9	175.1	175.3	175.5	175.7	175.9	176.1	176.3	176.5	176.7	176.9	177.1	177.3	177.5	177.7	177.9	178.1	178.3	178.5	178.7	178.9	179.1	179.3	179.5	179.7	179.9	180.1	180.3	180.5	180.7	180.9	181.1	181.3	181.5	181.7	181.9	182.1	182.3	182.5	182.7	182.9	183.1	183.3	183.5	183.7	183.9	184.1	184.3	184.5	184.7	184.9	185.1	185.3	185.5	185.7	185.9	186.1	186.3	186.5	186.7	186.9	187.1	187.3	187.5	187.7	187.9	188.1	188.3	188.5	188.7	188.9	189.1	189.3	189.5	189.7	189.9	190.1	190.3	190.5	190.7	190.9	191.1	191.3	191.5	191.7	191.9	192.1	192.3	192.5	192.7	192.9	193.1	193.3	193.5	193.7	193.9	194.1	194.3	194.5	194.7	194.9	195.1	195.3	195.5	195.7	195.9	196.1	196.3	196.5	196.7	196.9	197.1	197.3	197.5	197.7	197.9	198.1	198.3	198.5	198.7	198.9	199.1	199.3	199.5	199.7	199.9	200.1	200.3	200.5	200.7	200.9	201.1	201.3	201.5	201.7	201.9	202.1	202.3	202.5	202.7	202.9	203.1	203.3	203.5	203.7	203.9	204.1	204.3	204.5	204.7	204.9	205.1	205.3	205.5	205.7	205.9	206.1	206.3	206.5	206.7	206.9	207.1	207.3	207.5	207.7	207.9	208.1	208.3	208.5	208.7	208.9	209.1	209.3	209.5	209.7	209.9	210.1	210.3	210.5	210.7	210.9	211.1	211.3	211.5	211.7	211.9	212.1	212.3	212.5	212.7	212.9	213.1	213.3	213.5	213.7	213.9	214.1	214.3	214.5	214.7	214.9	215.1	215.3	215.5	215.7	215.9	216.1	216.3	216.5	216.7	216.9	217.1	217.3	217.5	217.7	217.9	218.1	218.3	218.5	218.7	218.9	219.1	219.3	219.5	219.7	219.9	220.1	220.3	220.5	220.7	220.9	221.1	221.3	221.5	221.7	221.9	222.1	222.3	222.5	222.7	222.9	223.1	223.3	223.5	223.7	223.9	224.1	224.3	224.5	224.7	224.9	225.1	225.3	225.5	225.7	225.9	226.1	226.3	226.5	226.7	226.9	227.1	227.3	227.5	227.7	227.9	228.1	228.3	228.5	228.7	228.9	229.1	229.3	229.5	229.7	229.9	230.1	230.3	230.5	230.7	230.9	231.1	231.3	231.5	231.7	231.9	232.1	232.3	232.5	232.7	232.9	233.1	233.3	233.5	233.7	233.9	234.1	234.3	234.5	234.7	234.9	235.1	235.3	235.5	235.7	235.9	236.1	236.3	236.5	236.7	236.9	237.1	237.3	237.5	237.7	237.9	238.1	238.3	238.5	238.7	238.9	239.1	239.3	239.5	239.7	239.9	240.1	240.3	240.5	240.7	240.9	241.1	241.3	241.5	241.7	241.9	242.1	242.3	242.5	242.7	242.9	243.1	243.3	243.5	243.7	243.9	244.1	244.3	244.5	244.7	244.9	245.1	245.3

Annex B (informative)

Determination of caffeine content

B.1 Principle

Carbon dioxide is removed from the sample by means of dry air or dry nitrogen. An extraction with chloroform is performed on the decarbonated sample.

By means of a graph of absorbance against concentration of standards, the content of caffeine of the sample is determined.

B.2 Reagents

During analysis use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

- a) Chloroform
- b) Ammonia solution, concentrated
- c) Hydrochloric acid, approximately molar. Take 100 ml of concentrated hydrochloric acid (sp. gr. 1.184) and dilute to a litre.
- d) Standard caffeine solution. Prepare a solution containing 10 ml using molar hydrochloric acid as solvent.

B.3 Apparatus

A spectrophotometer or photoelectric colorimeter capable of measuring optical density at a wavelength of 227 nm.

B.4 Procedure

Transfer 25 g of decarbonated sample into a small separating funnel. Make distinctly alkaline with ammonia solution and chloroform, washing each extract with the same 10 ml of water contained in a second separating funnel, and finally with the extract once with 10 ml of chloroform. Filter into a small flask. Evaporate or distil the combined extracts and dry the residue in molar hydrochloric acid and make up to volume in a 50 ml volumetric flask with the same acid. Prepare a series of standards and read the absorption at 272 nm using approximately molar hydrochloric acid for setting the instrument. Set the instrument by means of a blank prepared from water treated in exactly the same manner with the test solution and read absorption of the test solution.

B.5 Expression of results

Plot a graph of concentrations of standard caffeine solutions against their absorbance. From this graph determine the concentration of the alkaloid (caffeine) in the original sample. Report the results as mg/kg of anhydrous caffeine in the original sample.

Annex D (informative)

Determination of quinine

D.1 Principle

- Carbon dioxide is removed from the sample by passing through it dry air or dry nitrogen. An extraction with ether is performed on the decarbonated sample.
- By means of a graph of concentration of a series of standard quinine sulphate solutions against fluorescence, the content of quinine in the test solution is determined.

D.2 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity. Reagents should be free from fluorescing impurities.

- a) Sulphuric acid, 0.05 M
- b) Ammonia solution, concentrated
- c) Diethyl ether
- d) Quinine sulphate, standard stock solution. Dissolve 0.10 g of quinine sulphate in 0.05 M sulphuric acid and make up to 1 litre with 0.05 M sulphuric acid. This solution contains 100 micrograms of quinine sulphate per millilitre.
- e) Quinine sulphate, standard working solution. Dilute 10 ml of the quinine sulphate stock solution to 200 ml with 0.05 m sulphuric acid. This solution contains 1 microgram of quinine sulphate per millilitre.

D.3 Apparatus

An instrument capable of measuring fluorescence. Note that glassware should completely be free from stopcock lubricant as this usually contains fluorescence substances. No detergents shall be used in washing glassware.

D.4 Procedure

Transfer 100 g of the decarbonated sample to a separating funnel. Make the sample distinctly alkaline with ammonia solution and extract with the same 10 ml of water contained in a second separating funnel, and finally extract the wash water once with 10 ml of diethyl ether. Combine the ether extracts and remove the ether by distillation. Dry the residue in an air oven at 100 °C for a few minutes. Dissolve it in 0.05 M sulphuric acid and make up to 100 ml in a volumetric flask with 0.05 m sulphuric acid. Dilute 10 ml of this solution to 200 ml with 0.05 m sulphuric acid. Measure the fluorescence of the solution by means of a suitable instrument. Prepare a series of standards containing 0.1, 2.5 and 10 micrograms of quinine sulphate per millilitre and measure the fluorescence.

D.5 Expression of results

Plot the fluorescence results of the series of standards to obtain a curve from which the concentration of quinine in the test solution can be read. Calculate the concentration of quinine as mg/kg quinine sulphate in the original sample.

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