



DRAFT EAST AFRICAN STANDARD

Water-based flavoured drinks — Specification — Part 1: Carbonated and non-carbonated soft drinks

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 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 34

This Second edition cancels and replaces EAS 29: 2000, which has been technically revised.

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.

EAS 29 consists of the following parts, under the general title *Water-based flavoured drinks — Specification*:

- *Part 1: Carbonated and non-carbonated soft drinks*
- *Part 2: Energy drinks*

Water-based flavoured drinks — Specification — Part 1: Carbonated and non-carbonated soft drinks

1 Scope

This Draft East African Standard specifies the requirements, sampling, and test methods for carbonated and non-carbonated soft drinks (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

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 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, *General principles of food hygiene — Code of practice*

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/obp>

— IEC Electropedia: available at <http://www.electropedia.org/>

**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**
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
hermetically sealed containers**
sealed containers designed to be completely airtight, protecting the contents from the entry of microorganisms, moisture, contaminants and air throughout the product's shelf life.

3.5

food grade packaging

Packaging which safeguards the hygienic, nutritional, technological and organoleptic qualities of the product and does 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 or without permitted food additives. Includes gaseosa (water-based drinks with added carbon dioxide, sweetener, and flavour), and sodas such as colas, pepper-types, root beer, lemon-lime, and citrus types, both diet/light and regular types. These beverages may be clear, cloudy, or may contain particulated matter (e.g. fruit pieces).

4.2.2 non-carbonated soft drinks

Water-based flavoured drinks without added carbon dioxide, with or without the addition of fruit and vegetable juice-based drinks, milk or milk solids, fruit-flavoured ades and punches, capile groselha, lactic acid beverage, and herbal-based drinks. This may include "sports" drinks containing electrolytes. These beverages may be clear or contain particulate matter with or without permitted food additives and may be unsweetened or sweetened.

5 Requirements

5.1 Composition requirements

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

- a) water complying with the requirements of EAS 12;
- b) carbon dioxide complying with the requirements of EAS 149;
- c) sweetening agents;
- d) edible common salt complying with the requirements of EAS 35;
- e) for the purposes of product fortification, essential nutrients such as vitamins and minerals may be added to carbonated and non-carbonated soft drinks;
- f) fruit or vegetable juice, pulp, puree, vegetable extracts, herbal extracts or extracts from other plant parts or their blends complying with relevant standards;
- g) flavouring agents in accordance with CXG 66;
- h) quinine salts, if used shall comply with requirements given in Table 1; and
- i) caffeine, 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 off-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; and
- h) show no sedimentation except in particulate drinks that contain particles such as pieces of fruits or vegetables.

6 Specific requirements

Carbonated and non-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	Parameter	Requirement	Test method
i.	pH, min	2.5	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 C
v.	Total soluble solids, degree Brix (°Brix) at 20 °C, a) Sugar containing drinks, min b) Low calorie drink, max c) sugar free drink, max	5.1 5 0.5	ISO 2173
vi.	Carbon dioxide, CO ₂ , min. (for carbonated soft drinks)	One gas volume ^a	Annex A
^a 1 volume of carbonation equals to 1.97667 g/l.			

7 Food additives

Food additives, when used in Carbonated and non-carbonated soft drinks, shall comply with CXS 192.

8 Contaminants

8.1 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.

8.2 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	Limit	Test method
i.	Arsenic (As), mg/kg, max.	0.1	ISO 6634
ii.	Lead (Pb), mg/kg, max.	0.1	ISO 6633
iii.	Tin (Sn), mg/kg (for canned products), max.	150	ISO 2447
iv.	Cadmium (Cd) mg/kg, max.	0.003	AOAC 999.10

9 Hygiene

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

9.2 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	Test method
i.	Total plate count, cfu/per ml, max.	100	ISO 4833-1 ISO 4833-2
ii.	Coliform count, cfu/ml,	Absent	ISO 4832
iii.	Yeast and mould count, cfu/ml, max.	30	ISO 21527-1

10 Labelling

10.1 In addition to the requirements of EAS 38, each container, cap or label shall be legibly and indelibly labelled with the following information:

- a) name of product
- b) List of ingredients in descending order;

- c) name and physical address of the manufacturer, packer, distributor, importer, exporter or vendor;
- d) net content
- e) date of manufacture in code or in clear;
- f) expiry, use-by or best before date; and
- g) batch/lot number.
- h) Allergen declaration where applicable
- i) Instructions for use where applicable

10.2 In the case the product is carbonated, the word "carbonated" shall appear near the name of the product.

10.3 Use of pictorial representation or the use of the words contains "fruit juice" on carbonated and non-carbonated soft drinks on label is prohibited except where the species of fruits or fruit juices present in the drink is in amount constituting minimum 10% or where two or more fruits or fruit juices are used, the combination of the fruits or fruit juices whose contents constitutes minimum 10%

11. Nutrition labelling and claims

11.1 Nutrition labelling shall be done in accordance with EAS 803.

11.2 Nutrition and health claims, if used, shall be in accordance with EAS 804 and EAS 805 as appropriate

12 Sampling

Sampling shall be done in accordance with CXG 50.

Annex A (normative)

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 A. 2.1

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Caloric Pressure in kg/cm ² —	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
Temperature °C	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52
0	1.71	1.69	2.21	2.4	2.6	2.9	3.1	3.3	3.5	3.7	4.0	4.2	4.4	4.6	4.8	5.1	5.4	5.6	5.9	6.1	6.3	6.5	6.7	7.0	7.2	7.4	7.7
1	1.68	1.67	2.19	2.3	2.5	2.7	2.9	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8
2	1.64	1.63	2.15	2.2	2.4	2.6	2.7	2.9	3.1	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6
3	1.57	1.56	2.08	2.1	2.3	2.4	2.6	2.7	2.9	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4
4	1.54	1.53	2.05	2.0	2.2	2.3	2.5	2.6	2.8	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
5	1.51	1.50	2.02	1.9	2.1	2.2	2.4	2.5	2.7	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2
6	1.47	1.46	1.99	1.8	2.0	2.1	2.3	2.4	2.6	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
7	1.43	1.42	1.95	1.7	1.9	2.0	2.2	2.3	2.5	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0
8	1.39	1.38	1.91	1.6	1.8	1.9	2.1	2.2	2.4	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
9	1.35	1.34	1.87	1.5	1.7	1.8	2.0	2.1	2.3	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
10	1.31	1.30	1.83	1.4	1.6	1.7	1.9	2.0	2.2	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
11	1.27	1.26	1.79	1.3	1.5	1.6	1.8	1.9	2.1	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6
12	1.23	1.22	1.75	1.2	1.4	1.5	1.7	1.8	2.0	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
13	1.19	1.18	1.71	1.1	1.3	1.4	1.6	1.7	1.9	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4
14	1.15	1.14	1.67	1.0	1.2	1.3	1.5	1.6	1.8	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
15	1.11	1.10	1.63	0.9	1.1	1.2	1.4	1.5	1.7	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2
16	1.07	1.06	1.55	0.8	1.0	1.1	1.3	1.4	1.6	1.7	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
17	1.03	1.02	1.51	0.7	0.9	1.0	1.2	1.3	1.5	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0
18	0.99	0.98	1.47	0.6	0.8	0.9	1.1	1.2	1.4	1.5	1.7	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
19	0.95	0.94	1.43	0.5	0.7	0.8	1.0	1.1	1.3	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8
20	0.91	0.90	1.39	0.4	0.6	0.7	0.9	1.0	1.2	1.3	1.5	1.7	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
21	0.87	0.86	1.35	0.3	0.5	0.6	0.8	0.9	1.1	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
22	0.83	0.82	1.31	0.2	0.4	0.5	0.7	0.8	1.0	1.1	1.3	1.5	1.7	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
23	0.79	0.78	1.27	0.1	0.3	0.4	0.6	0.7	0.9	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4
24	0.75	0.74	1.23	0.0	0.2	0.3	0.5	0.6	0.8	0.9	1.1	1.3	1.5	1.7	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
25	0.71	0.70	1.19	-0.1	0.1	0.2	0.4	0.5	0.7	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2
26	0.67	0.66	1.15	-0.2	0.0	0.1	0.3	0.4	0.6	0.7	0.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1
27	0.63	0.62	1.11	-0.3	-0.1	0.0	0.2	0.3	0.5	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
28	0.59	0.58	1.07	-0.4	-0.2	-0.1	0.1	0.2	0.4	0.5	0.7	0.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9
29	0.55	0.54	1.03	-0.5	-0.3	-0.2	0.0	0.1	0.3	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8
30	0.51	0.50	0.99	-0.6	-0.4	-0.3	0.1	0.2	0.4	0.5	0.7	0.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9
31	0.47	0.46	0.95	-0.7	-0.5	-0.4	0.2	0.3	0.5	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
32	0.43	0.42	0.91	-0.8	-0.6	-0.5	0.3	0.4	0.6	0.7	0.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1
33	0.39	0.38	0.87	-0.9	-0.7	-0.6	0.4	0.5	0.7	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2
34	0.35	0.34	0.83	-1.0	-0.8	-0.7	0.5	0.6	0.8	0.9	1.1	1.3	1.5	1.7	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
35	0.31	0.30	0.79	-1.1	-0.9	-0.8	0.6	0.7	0.9	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4
36	0.27	0.26	0.75	-1.2	-1.0	-0.9	0.7	0.8	1.0	1.1	1.3	1.5	1.7	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
37	0.23	0.22	0.71	-1.3	-1.1	-1.0	0.8	0.9	1.1	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
38	0.19	0.18	0.67	-1.4	-1.2	-1.1	0.9	1.0	1.2	1.3	1.5	1.7	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
39	0.15	0.14	0.63	-1.5	-1.3	-1.2	1.0	1.1	1.3	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8
40	0.11	0.10	0.59	-1.6	-1.4	-1.3	1.1	1.2	1.4	1.5	1.7	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
41	0.07	0.06	0.55	-1.7	-1.5	-1.4	1.2	1.3	1.5	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0
42	0.03	0.02	0.51	-1.8	-1.6	-1.5	1.3	1.4	1.6	1.7	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
43	-0.01	-0.02	0.47	-1.9	-1.7	-1.6	1.4	1.5	1.7	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2
44	-0.05	-0.06	0.43	-2.0	-1.8	-1.7	1.5	1.6	1.8	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
45	-0.09	-0.10	0.39	-2.1	-1.9	-1.8	1.6	1.7	1.9	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4
46	-0.13	-0.14	0.35	-2.2	-2.0	-1.9	1.7	1.8	2.0	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
47	-0.17	-0.18	0.31	-2.3	-2.1	-2.0	1.8	1.9	2.1	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6
48	-0.21	-0.22	0.27	-2.4	-2.2	-2.1	1.9	2.0	2.2	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
49	-0.25	-0.26	0.23	-2.5	-2.3	-2.2	2.0	2.1	2.3	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
50	-0.29	-0.30	0.19	-2.6	-2.4	-2.3	2.1	2.2	2.4	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
51	-0.33	-0.34	0.15	-2.7	-2.5	-2.4	2.2	2.3	2.5	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4</			

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Annex B (normative)

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

B.2.1 During analysis use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

B.2.1.1 Chloroform

B.2.1.2 Ammonia solution, concentrated

B.2.1.3 Hydrochloric acid, approximately molar. Take 100 ml of concentrated hydrochloric acid (sp. gr. 1.184) and dilute to a litre

B.2.1.4 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 C (normative)

Determination of quinine

C.1 Principle

C.1.1 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.

C.1.2 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.

C.2 Reagents

C.2.1 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.

C.2.1.1 Sulphuric acid, 0.05 M

C.2.1.2 Ammonia solution, concentrated

C.2.1.3 Diethyl ether

C.2.1.4 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.

C.2.1.5 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.

C.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.

C.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.

C.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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