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Bee propolis — Specifications

Propolis d'abeille — Spécifications

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 19, *Bee products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Propolis is a resinous substance produced by worker bees combining plant resins and/or fragments of newly formed buds with their salivary and wax gland secretions.

The chemical composition of propolis is quite complex. Hundreds of natural compounds such as flavonoids and phenolic acids have been identified in propolis. Different geographical and plant sources, bee species, production methods, etc., have a significant influence on the chemical composition of propolis.

For the purposes of this document, propolis is divided into *Populus, Baccharis* and *Dalbergia* types (the primary sources) and opens the opportunity in the future to cover other types, such as *Araucaria* spp., *Betula* spp., *Castanea* spp., *Clusia* spp., *Cupressus* spp., *Eucalyptus* spp., *Macaranga* spp., *Symphonia* spp., and the mixed plant source propolis (this list is not exhaustive). Only propolis produced by *Apis mellifera* bees is covered in this document.

Scientific literature predominantly relates to three main propolis types (brown, green and red) of which brown (*Populus*) and green (*Baccharis*) propolis are the main types traded internationally. This document considers the complex chemical composition of propolis, and the influences that geographical and plant species variation, and honey bee sub-species have on the proximate, flavonoid and phenolic composition of propolis. Propolis is rich in polyphenols, in particular flavonoids, phenolic acids and derivatives, which can be involved in the biological activities. The decisions made about the types, methodologies and requirements included in this document were based on the scientific literature available at the time.

This document sets out the terms, definitions, classification, quality requirements, authenticity requirements, test method procedures, transportation, storage conditions and packing marks. It aims to provide a document for the classification and quality control for the international trade of raw propolis.

Bee propolis — Specifications

1 Scope

This document specifies the quality requirements, analytical methods, and packaging, marking, labelling, storage and transportation conditions for bee propolis.

This document is applicable to propolis collected from beehives of *Apis mellifera* colonies, i.e. raw propolis.

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.

ISO 22005, *Traceability in the feed and food chain — General principles and basic requirements for system design and implementation*

3 Terms and definitions

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
antioxidant capacity
ability of a substance that retards deterioration of oxidation

3.2
ash content
incombustible component remaining after a sample of *raw propolis* (3.17) is completely burnt

3.3
authenticity requirement
requirement that the addition of resins, extracts or any compounds, and/or bioactive substances in raw propolis is not allowed

3.4
balsamic
relating to or containing balsam

3.5
beeswax
honey bee secretions including mixtures of substituted long-chain aliphatic hydrocarbons, containing alkanes, alkyl esters, fatty acids, primary and secondary alcohols, diols, ketones and aldehydes

3.6
contaminant
substance not intentionally added to propolis, which is present as a result of a process such as production to harvesting, manufacture, processing, preparation, treatment, packing, packaging, transport or holding of the product, or because of environmental contamination

3.7
ethanol extractables of propolis (as dry matter)
content obtained after an exhaustive extraction process using ethanol (80 % volume/volume) as the extractor solvent

Note 1 to entry: Also known as genuine, crude, balsamic and dry extract of propolis.

3.8
petroleum ether extractables of propolis (as dry matter)
content obtained after an exhaustive extraction process using petroleum ether as an extraction solvent

3.9
flavonoids
class of plant and fungus secondary metabolites that have the general structure of a 15-carbon skeleton (abbreviated C6-C3-C6), consisting of two phenyl rings (A and B) and a heterocyclic ring (C) that includes subgroups of flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones

3.10
harvesting
mechanical process used to remove raw propolis from the hive where it is deposited by the *Apis mellifera*

3.11
loss on drying
amount of all volatile substances present in propolis sample, including moisture, that is lost in drying at 105 °C for 1 h

3.12
phenolic acids
carboxylic acids derived from either benzoic or cinnamic acid skeletons

3.13
plants of propolis source
plants of different geographical and botanical origin, which produce resinous balsamic exudates that are collected and converted into *propolis* (3.15) in beehives by *Apis mellifera* bees

3.14
polyphenols
natural organic molecules widely present in the plant kingdom, which are the main active ingredients of propolis, and are characterized by the presence of multiple phenolic groups associated in complex structures, some of them with high molecular mass

3.15
propolis
resinous balsamic mixture, exclusively of natural and plant origin, harvested by worker bees of the species *Apis mellifera* from newly formed buds, flower buds and exudates of specific *plants of propolis source* (3.13), to which the bees add their own secretions, mostly from their salivary and wax glands, and use it to protect the honey bee and colony health

3.16
propolis extract
components, derived from *raw propolis* (3.17) devoid of foreign matter, that are soluble in solvents generally recognized as safe (GRAS) for human consumption

3.17
raw propolis
propolis produced by *Apis mellifera* without any external intervention, except the removal from the hive

3.18
total phenolic content
total amount of any compound with a hydroxyl group linked directly to a benzene ring

3.19
traceability
ability to follow the movement of propolis through specified stage(s) of production, processing and distribution

4 Requirements

4.1 Classification of raw propolis types

4.1.1 Temperate, Mediterranean and boreal, brown, *Populus spp.* propolis

Poplar propolis can be brownish yellow, brownish red, brown, yellowish-brown, greyish-brown, greenish-brown or grey-black. The main botanical source is *Populus spp.* However, several other botanical sources can be present. At 20 °C to 24 °C, it appears as a lump shape or broken grains shape, and becomes soft, malleable and sticky with the increase of temperature above 30 °C. There is a characteristic balsamic and resinous aroma of Poplar propolis. The taste is slightly bitter, a little astringent and micro-tingly.

4.1.2 Tropical, green, *Baccharis dracunculifolia* propolis

Baccharis propolis is yellowish-green, green, greenish, greenish-brown and brown. The main botanical source is *Baccharis dracunculifolia*. At 20 °C to 24 °C, it appears strip shape and broken grains shape, and becomes malleable at about 25 °C. It has a characteristic resinous, woody, spicy aromatic odour, and a strong bitter and spicy taste.

4.1.3 Tropical, red, Dalbergia and Clusia propolis

Dalbergia and *Clusia* propolis are red, yellowish-red and brownish-red. The main botanical sources are *Dalbergia ecastaphyllum*, *Symphonia globulifera* and *Clusia* spp. At temperatures higher than 20 °C they appear malleable. It has a characteristic resinous and aromatic odour. The taste is aromatic and slightly bitter.

4.1.4 Other types of propolis

The current scientific literature does not provide complete data to fully characterize the chemical and biological properties of other floral types of propolis for inclusion in this document. Some examples of other types of propolis are *Araucaria spp., Betula spp., Castanea spp.*, Cupressaceae family, *Macaranga tanarius*, Salicaceae, Pinaceae and others (the list is not exhaustive).

4.2 Physical and chemical requirements

The physical and chemical requirements of propolis shall be as given in Table 1, except total flavonoids that are a normative parameter, but the corresponding procedure shall be selected between Annex F or G.

Table 1 — Physical and chemical requirements for bee propolis and test methods for each characteristic

Characteristic	Min. or max.	Requirements (on a dry basis)			Test method
		Brown propolis (4.1.1)	Green propolis (4.1.2)	Red propolis (4.1.3)	
Ethanol extractables of propolis (as dry matter), in % mass fraction	min.	30,0	30,0	30,0	Annex A
Loss on drying, in % mass fraction	max.	10,0	10,0	10,0	Annex B
Ash content, in % mass fraction	max.	5,0	5,0	5,0	Annex C
Petroleum ether extractables of propolis (as dry matter), in % mass fraction	max.	65,0	30,0	60,0	Annex D
Total phenolic compounds (Folin), in % mass fraction, as gallic acid[a]	min.	10,0	7,0	7,0	Annex E
Total phenolic compounds (Folin), in % mass fraction, as galangin[a]	min.	17,0	12,0	12,0	Annex E
Total flavonoids ($AlCl_3$), in % mass fraction, as quercetin	min.	3,0	1,0	0,5	Annex F
Total flavonoids (polyamide method), in % mass fraction, as rutin	min.	6,0	2,0	1,0	Annex G
Total polyphenolics by high-performance liquid chromatography (HPLC) (poplar, green and red propolis)	—	Presence of: apigenin, caffeic acid, CAPE, p-coumaric acid, chrysin, ferulic acid, galangin, pinobanksin and pinocembrin	Presence of: caffeic acid, p-coumaric acid, 3,5-dicaffeoyilquinic acid, 4,5-dicaffeoyilquinic acid, cinnamic acid, drupanin, artepellin C and baccharin	Presence of: calycosin, isoliquiritigenin, formononetin and biochanin	Annex H Annex I Annex J
Total antioxidant capacity (DPPH) – EC50, in µg/ml	max.	25,0	40,0	50,0	Annex K

[a] Total phenolic can be expressed as gallic acid or galangin equivalents. To convert from gallic acid to galangin, multiply the value obtained using the conversion factor of 1,7. To convert from galangin to gallic acid, multiply the value by 0,59.

4.3 Traceability requirements

ISO 22005 shall be followed to guarantee the traceability of propolis.

5 Test methods

5.1 Reagents

Use only analytical grade reagents unless otherwise specified. Distilled water should be in accordance with the first-grade water or water with the same purity given in ISO 3696.

5.2 Sample collection

Propolis is a very heterogeneous product; therefore, at least 1 % of the batch (minimum of 1 kg of sample for batches less than 100 kg) shall be collected. A minimum of 10 representative points shall be sampled to take into consideration the diversity of the propolis. Pack them in a food-grade container and store them below –18 °C.

Sampling tools shall be clean and shall not add any foreign matter or contaminants to the samples.

5.3 Sample preparation

Combine the representative points samples and crush in a pulveriser, while still frozen, until they pass through a 10 mesh (2 mm) sieve. If there are visible impurities before the pulverization, they shall be removed. Transfer to a food-grade container and mix until homogenous. Take an appropriate subsample sufficient for testing, seal in an airtight container and store at below –18 °C, if required, until analysis.

5.4 Test methods for physical and chemical requirements

The sample should be tested according to the test methods specified in Annexes A to K.

6 Packaging, marking, labelling, storage and transportation

6.1 Packaging

Raw propolis packaging should protect the product from light. Propolis loss on drying shall be lower than 10 %.

6.2 Marking (label and/or certificate)

The information listed in Table 2 shall be used on each package or label/certificate. Additional information can be included.

6.3 Labelling

Labelling requirements shall be as given in Table 2.

Table 2 — Labelling requirements

Requirement	Beekeeper	Company B2B	Company B2C
Product name and brand (if exists), and/or trademark	✓	✓	✓
Name, complete address of the producer and packer	✓	✓	✓
Net mass	✓	✓	✓
Country or countries of origin (in order of proportional content: highest to lowest)	✓	✓	✓
Propolis type according to 4.1 of this document	✓	✓	✓
Ethanol extractables of propolis[a]	—	—	✓

Key

B2B: business to business

B2C: business to consumers

[a] Raw propolis for consumers: The information described on the label, or accessed by certificate of analysis using a QR code, or other equivalent (see Annex A).

[b] In the case of total phenolic, identify if gallic acid or galangin equivalent is being used (see Annex E).

[c] In the case of total flavonoids, identify quercetin (see Annex F, $AlCl_3$) or rutin (see Annex G, polyamide) equivalents.

Table 2 *(continued)*

Requirement	Beekeeper	Company B2B	Company B2C
Harvesting time: month(s)/year(s)	✓	✓	—
Best before or expiry date	—	✓	✓
Storage information	—	✓	✓
Batch number	✓	✓	✓
Total phenolics[b]	—	—	✓
Total flavonoids[c]	—	—	✓

Key

B2B: business to business

B2C: business to consumers

[a] Raw propolis for consumers: The information described on the label, or accessed by certificate of analysis using a QR code, or other equivalent (see Annex A).

[b] In the case of total phenolic, identify if gallic acid or galangin equivalent is being used (see Annex E).

[c] In the case of total flavonoids, identify quercetin (see Annex F, $AlCl_3$) or rutin (see Annex G, polyamide) equivalents.

6.4 Storage and transportation

Storage and transportation shall take into consideration the type of propolis, protection from light, elevated temperature (keep < 25 °C) and humidity conditions of the room to avoid the degradation of the genuine characteristics of propolis and prevent the growth of microorganisms on the surface.

Raw propolis shall not be stored and shipped with articles that are odorous, poisonous and corrosive, and potentially polluting products.

Annex A
(normative)

Ethanol extractables of raw propolis (as dry matter)

A.1 Principle

Propolis is partially soluble in an ethanol/water solution. The dry mass of ethanol/water extract is calculated as a percentage of the mass of the sample, after complete removal of the solvent.

A.2 Reagents and materials

A.2.1 Ethanol, $\varphi(CH_3CH_2OH)$ = 80 % (volume/volume).

A.2.2 Iron (III) chloride in methanol = 5 % (mass/volume).

A.3 Apparatus and equipment

A.3.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

A.3.2 Vacuum dryer or oven.

A.3.3 Erlenmeyer or beaker, 100 ml.

A.3.4 Magnetic stirrer.

A.3.5 Glass funnel, Φ = 60 mm.

A.3.6 Quantitative filter paper, Φ = 12,5 cm.

A.3.7 Magnetic rod.

A.3.8 Volumetric flask, 100 ml

A.4 Procedure

Use the following procedure:

a) The extraction procedure given in steps b) to f) shall be done in triplicate.

b) Weigh 1 g (accurate to 0,001 g) of the propolis sample (m_1) into a 100 ml Erlenmeyer or beaker (A.3.3) and add 30 ml of 80 % ethanol.

c) Keep the mixture under mechanical or manual agitation, at 50 °C, for 3 h and protected from light.

d) Then, filter the mixture through quantitative filter paper.

e) To confirm the absence of phenolics in the remaining residue, add a few drops of $FeCl_3$ (5 % in methanol). If a colour development is observed, the residue shall be reextracted (following steps a) to c)), until no colour development is observed (no more than three extractions).

f) Combine all extracts in a 100 ml volumetric flask and make up to volume with ethanol 80 % volume/volume.

g) For evaluation of the dry extract, weigh a glass drying dish (m_2), combine 2 ml of each extraction solution (3 x 2) ml in a glass drying dish and dry to constant mass (m_3) in an oven set at 105 °C for 90 min. Place the dish in a desiccator at room temperature (15 min) between weighings (maximum of 2 mg of difference between two consecutive weighings).

A.5 Calculation

The content of ethanol/water extract in the sample (expressed as dry matter), E_E, expressed as a percentage of mass, is given by Formula (A.1):

$$E_E = \frac{m_3 - m_2}{m_1} \times \left(\frac{50}{3} \right) \times 100 \quad \text{(A.1)}$$

where

m_1 is the average mass of the propolis sample, in g;

m_2 is the mass of the glass drying dish, in g;

m_3 is the mass of the dry extract and glass drying dish, in g.

NOTE (50/3) is the dilution factor.

A.6 Precision

The relative deviation of parallel experiments shall not be more than 5 %.

Annex B
(normative)

Loss on drying determination

B.1 Principle

The determination of the loss on drying is the amount of all volatile substances present in the propolis sample, including moisture, that is lost on drying. The method is gravimetric, and the result is calculated as a % mass fraction.

B.2 Apparatus and equipment

B.2.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

B.2.2 Drying oven.

B.2.3 Glass, porcelain or metal crucible (capsule), with diameter not less than 90 mm.

B.2.4 Desiccator.

B.2.5 Sieve 12 mesh.

B.2.6 Crushing apparatus.

B.3 Procedure

B.3.1 Sample preparation

Place a quantity of propolis sample in the freezer for 3 h. Remove the sample and immediately crush it, until it passes through a sieve 12 mesh.

B.3.2 Analysis

Use the following procedure:

a) The procedure given in steps b) to f) shall be done in triplicate.

b) Dry the glass, porcelain, or metal crucible (capsule) in drying oven at 105 °C (±2 °C) for 1 h. Transfer to desiccator until room temperature and weigh (m_c).

c) Weigh 2,0 g (with 0,000 1 g accuracy) of the sample (m_i) and spread it well in the capsule.

d) Place the capsule with the sample into drying oven at 105 °C (±2 °C) for a minimum of 1 h.

e) Transfer the capsule with the sample to desiccator until it cools to room temperature.

f) Weigh the capsule with the sample (m_f) and repeat the procedure until a constant mass is achieved (maximum of 2 mg of difference between two consecutive weighings).

g) Perform the analysis in triplicate.

B.4 Calculation

The content of loss on drying, L_D, expressed as a percentage of mass, is given by Formula (B.1):

$$L_D = 100 - \left[\frac{m_f - m_C}{m_i} \times 100 \right] \quad \text{(B.1)}$$

where

m_i is the initial mass of the propolis sample, in g;

m_c is the mass of the capsule, in g

m_f is the final mass, after drying, in g

B.5 Precision

The relative deviation of parallel experiments shall not be more than 5 %.

Annex C
(normative)

Ash content in raw propolis

C.1 Principle

Ash content represents the incombustible component remaining after a sample of raw propolis is completely burnt. High values can indicate possible adulteration of the material through the presence of impurities, or even inorganic residues from harvesting procedures.

C.2 Apparatus and equipment

C.2.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

C.2.2 Muffle furnace.

C.2.3 Silica or platinum crucible.

C.2.4 Desiccator.

C.3 Procedure

Use the following procedure:

a) Heat a silica or platinum crucible to redness for 30 min in the muffle furnace, allow to cool in a desiccator and weigh (w_1).

b) Weigh 2 g of raw propolis (w_2) in the dried crucible previously weighed.

c) Place the crucible in the muffle furnace at 300 °C for 30 min to ignite the sample with small fire until it is smokeless, and the sample is carbonized.

d) Incinerate the sample in a muffle furnace at 600 °C during 3 h, or until white, pasty white or creamy coloured ashes are obtained.

e) Cool the crucible containing the ashed sample in a desiccator and weigh (w_3).

f) Repeat the incineration process (additional 30 min), cooling and weighing until a constant mass is achieved (maximum of 2 mg of difference between two consecutive weighings) (w_3).

C.4 Calculation

The content of ash in the sample, Y_1, expressed as a percentage, is given by Formula (C.1):

$$Y_1 = \frac{(w_3 - w_1)}{w_2} \times 100 \qquad \text{(C.1)}$$

where

w_1 is the mass of the silica or platinum crucible, in g;

w_2 is the mass of raw propolis sample, in g;

w_3 is the mass of ash and crucible, in g.

C.5 Precision

The relative deviation of parallel experiments shall not be more than 5 %.

Annex D
(normative)

Petroleum ether extractables of raw propolis (as dry matter)

D.1 Principle

Raw propolis contains beeswax as a natural constituent produced by the bees. Non-polar petroleum ether removes waxes and some other non-polar compounds.

D.2 Reagents

D.2.1 Petroleum ether.

D.3 Apparatus and equipment

D.3.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

D.3.2 Soxhlet apparatus, complete with a condenser and cooling system.

D.3.3 Cellulose cartridge.

D.3.4 Flat or round bottom flask, 250 ml.

D.3.5 Vacuum dryer (rotary evaporator).

D.3.6 Desiccator.

D.4 Procedure

Use the following procedure:

a) Weigh 2 g of raw propolis (w_4) in a cellulose cartridge.

b) Extract the sample with petroleum ether in a Soxhlet apparatus for 6 h.

c) Evaporate the extract to dryness under reduced pressure (at maximum 50 °C).

d) Leave the residue to cool in a desiccator until a constant mass is achieved (w_5).

D.5 Calculation

The content of wax in the sample (count by dry matter), Z_1, expressed as a percentage of mass, is given by Formula (D.1):

$$Z_1 = \frac{w_5}{w_4} \times 100 \qquad \text{(D.1)}$$

where

w_4 is the mass of raw propolis sample, in g;

w_5 is the mass of dry residue extract, in g.

D.6 Precision

The relative deviation of parallel experiments shall not be more than 5 %.

Annex E
(normative)

Total phenolic content

E.1 Principle

Propolis is a mixture of natural substances very rich in phenolic compounds such as phenolic acids, flavonoids and its derivatives. The Folin-Ciocalteu method is an indirect measure of the total phenolic content in natural samples. Although not specific to phenolic compounds but truly to all reducing compounds, when used on propolis extracts it is a quick and reliable procedure to obtain a representative value of the phenolic content, due to the minimum presence of other reducing interferences such as amino acids and reducing sugars. The total phenolic content is expressed in percentage of raw propolis using gallic acid as the calibration standard. Alternatively, gallangin can also be used as the reference standard.

E.2 Reagents and materials

E.2.1 Folin-Ciocalteu reagent 2N or 1 mol/l.

E.2.2 Ethanol, $\varphi(CH_3CH_2OH)$ = 80 % (volume/volume).

E.2.3 Sodium carbonate, 20 % (mass/volume) (around pH = 12). A fresh reagent shall be used. A stock solution can be used for up to five working days when stored at 20 °C to 25 °C. In the case of crystallization during this period, another fresh solution should be prepared.

E.2.4 Gallic acid (>99,0 %).

For the stock solution (1 000 µg/ml), weigh accurately 10,0 mg gallic acid, put in 10 ml volumetric flask, add ethanol 80 % volume/volume to dissolve it, then fill to the mark and shake.

NOTE Adjust according to the purity of the standard.

E.2.5 Galangin (>97,0 %).

For the stock solution (1 000 µg/ml), weigh accurately 10,0 mg galangin, put in 10 ml volumetric flask, add ethanol 80 % volume/volume to dissolve it, then fill to the mark and shake.

NOTE Adjust according to the purity of the standard

E.2.6 Distilled water.

E.3 Apparatus and equipment

E.3.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

E.3.2 Ultraviolet (UV)-visible spectrophotometer.

E.3.3 Micropipettes.

E.3.4 Volumetric flasks.

E.3.5 Falcon tubes (10 ml) or similar.

E.3.6 pH meter or pH ribbon.

E.4 Procedure

E.4.1 Working solution

The samples should be extracted in accordance with Annex A. Prepare a working solution by pipetting 1,5 ml of propolis extract solution, S_V, (0,5 ml of each of triplicate extract solution) to a 10 ml volumetric flask and fill to the mark with ethanol 80 %.

E.4.2 Quantification

Use the following procedure:

a) The procedure given in steps b) and c) is carried out in triplicate.

b) Mix, in a falcon tube, an aliquot of the working solution (0,2 ml) with 1,5 ml of water and 0,4 ml of the Folin-Ciocalteu reagent.

c) Add 0,600 ml of a sodium carbonate solution (20 %) to the mixture, and adjust the final volume (5 ml) by adding 2,3 ml of deionized water.

d) Prepare the blank in the same conditions as the samples, using 0,2 ml of ethanol 80 % instead of the working solution.

e) Keep the mixture in the dark for 30 min at room temperature and measure the absorbance at 760 nm.

f) For the quantification (A), a calibration curve of gallic acid should be prepared using the same procedure as for the samples at least five points at the following final concentrations (reaction flask): 1,0 µg/ml; 2,0 µg/ml; 4,0 µg/ml; 6,0 µg/ml; 8,0 µg/ml – and apply the linear regression to have the equation to calculate "c" ($r^2 > 0{,}990$).

g) For the quantification (B), a calibration curve of galangin should be prepared using the same procedure as for the samples at least five points at the following concentrations (reaction flask): 1,0 µg/ml; 2,0 µg/ml; 4,0 µg/ml; 6,0 µg/ml; 8,0 µg/ml – and apply the linear regression to have the equation to calculate "c" ($r^2 > 0{,}990$).

h) If the sample absorbance does not fit within the calibration curve, the concentration of the working solution should be adapted.

E.5 Calculation

The content of total phenolics in the sample, P_1, expressed as % mass fraction, is given by Formula (E.1):

$$P_1 = \frac{c}{M \times S_v \times 40} \times 100 \qquad \text{(E.1)}$$

where

c is the concentration of the total phenolics expressed as gallic acid or galangin (according to "f" or "g") in the sample solution obtained by the calibration curve, in µg/ml;

S_v is the volume of the sample extract used to prepare the working solution (1,5 ml or other, according to the propolis type or quality);

M is the mean mass value of raw propolis (the mass of raw propolis used in the extraction of Annex A), in g.

E.6 Precision

The relative deviation of parallel experiments shall not be more than 10 %.

Annex F
(Normative)

Total flavonoids content (aluminium chloride method)

F.1 Principle

The flavonoids can combine with the chromogenic reagent Al^{3+} to form a coloured substance, which has the maximum absorption near 425 nm wavelength. Within a certain concentration range, the content of flavonoids is proportional to the absorbance. Compared with the standard curve, the content of flavonoids can be quantitatively measured.

F.2 Reagents and materials

F.2.1 Methanol (analytical grade).

F.2.2 Aluminium chloride solution, 5 % (mass/volume).

Weigh 5,00 g $AlCl_3$ (133,34 g/mol), dissolve in methanol, constant volume, in a 100 ml volumetric flask and shake.

F.2.3 Standard solution of quercetin.

F.2.3.1 Stock solution, 3 000 µg/ml.

Weigh accurately 30 mg of quercetin, put in 10 ml volumetric flask, add ethanol 80 % volume/volume to dissolve it, then fill to the mark and shake.

NOTE Adjust the mass according to the purity of the standard.

F.2.3.2 Prepare the dilutions of the reference standard.

Use ethanol 80 % volume/volume to achieve the concentrations of 25 µg/ml, 75 µg/ml, 125 µg/ml, 175 µg/ml and 225 µg/ml.

F.3 Apparatus and equipment

F.3.1 UV-visible spectrophotometer.

F.3.2 Agitation mechanical.

F.4 Procedure

F.4.1 Propolis sample preparation

The samples should be extracted in accordance with Annex A. Prepare a working solution by pipetting 1,5 ml of propolis extract solution, S_V (0,5 ml of each of triplicate extract solution) into a 10 ml volumetric flask and fill to the mark with ethanol 80 %.

F.4.2 Quantification

Use the following procedure:

a) The procedure given in steps b) and c) is carried out in triplicate.

b) Mix, in a 25 ml volumetric flask, an aliquot of the working solution (1,0 ml) with 15 ml of methanol.

c) Add 0,5 ml of an aluminium chloride solution to the mixture and adjust the final volume (25 ml) with methanol.

d) Prepare a blank by adding 15 ml of methanol, 1,0 ml of ethanol 80 % volume/volume and 0,5 ml of aluminium chloride solution (F.2.2) into a 25 ml volumetric flask. Mix the contents by shaking, add methanol to the mark and shake it again.

e) Keep the mixture in the dark for 30 min at room temperature and measure the absorbance at 425 nm.

f) For the quantification, prepare a calibration curve of quercetin at final reaction flask concentrations of 1,0 µg/ml, 3,0 µg/ml, 5,0 µg/ml, 7,0 µg/ml and 9,0 µg/ml using the same procedure as for samples – and apply the linear regression to have the equation to calculate "cs" (r^2>0,990).

g) Calculate the content of flavonoids in the sample by the standard curve.

h) If the sample absorbance does not fit within the calibration curve, the concentration of the working solution should be changed.

F.5 Calculation

The content of total flavonoid content in the raw propolis, F_1, expressed as a percentage of mass (calculated as quercetin), is given by Formula (F.1):

$$F_1 = \frac{c_s}{m_1 \times S_v \times 40} \times 100 \quad \text{(F.1)}$$

where

c_s is the concentration of total flavonoids expressed as quercetin in the sample solution calculated from the standard curve in µg/ml

S_V is the volume of the sample extract used to prepare the working solution (1,5 ml or other, according to the propolis type or quality);

m_1 is the mass value of raw propolis (the mean mass of raw propolis used in the extraction of Annex A), in g.

F.6 Precision

The relative deviation of parallel experiments shall not be more than 10 %.

Annex G
(Normative)

Total flavonoids content (polyamide method)

G.1 Principle

Total flavonoids are extracted from the propolis with an ethanol/water solution. The flavonoids are isolated on a polyamide solid-phase column, eluted with methanol, and quantified against a rutin standard by measuring their absorbance at 360 nm.

G.2 Reagents and materials

G.2.1 Polyamide powder for column chromatography, particle size: approximately 100 mesh to 200 mesh.

G.2.2 Ethanol, $\varphi(CH_3CH_2OH)$: 80 % (volume/volume).

G.2.3 Methanol.

G.2.4 Toluene.

G.2.5 Rutin reference standard, purity ≥ 98 % (certificate of analysis of supplier).

G.3 Apparatus and equipment

G.3.1 Analytical balance, capable of accurately weighing to the nearest 0,000 1 g.

G.3.2 UV-visible spectrophotometer.

G.3.3 Glass column chromatography, 30 cm to 40 cm (long) × 1,0 mm to 1,2 mm (id).

G.3.4 Diagram of column chromatography device, see Figure G.1.

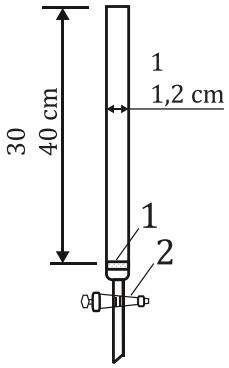
G.3.5 Measuring flasks (A grade), 10 ml, 50 ml, 100 ml.

G.3.6 Automatic pipettor, 100 µl to 1 000 µl (for accurate transfer of propolis diluent).

G.3.7 Manual glass pipet (A grade), 1 ml to 5 ml (for diluting standard solution).

G.3.8 Glass pan, Φ = 90 mm.

G.3.9 Quartz cuvette, optical path 1 cm.



Key

1 P70 sand core (aperture: (50-70) µm

2 PTFE or glass valve

NOTE

Figure G.1 — Diagram of glass column chromatography device

G.4 Procedure

G.4.1 Preparation of standards

G.4.1.1 Rutin stock solution (500 µg/ml)

Use a four-decimal balance to weigh 25 mg of rutin (G.2.5) into a small beaker. Record the actual mass to 0,000 1 g accuracy. Dissolve in methanol, and then quantitatively transfer into a 50 ml volumetric flask and adjust to the 50 ml mark with methanol. Mix thoroughly by inverting the flask repeatedly. This solution is stable for one year at −20 °C.

G.4.1.2 Rutin working standard solution (50 µg/ml)

Transfer 2,00 ml rutin stock solution (G.4.1.1) into a 20 ml volumetric flask and make up to the volume mark with methanol. Mix thoroughly by inverting the flask repeatedly. This solution is stable for one week at < +4 °C.

G.4.1.3 Rutin calibration standard solution (range 5 to 25 µg/ml)

Transfer the following volumes of rutin working standard solution (G.4.1.2) into individual 10 ml calibrated volumetric flasks and fill to the 10 ml mark with methanol (see Table G.1). Prepare fresh daily.

Table G.1 — Preparation of rutin calibration standard solution

Concentration[a] µg/ml	Volume of rutin working standard solution ml	Volume of methanol ml
0	0,00	10,00
5	1,00	9,00
10	2,00	8,00
15	3,00	7,00
20	4,00	6,00
25	5,00	5,00

[a] The actual concentration should be calculated based on the accurate mass of the stock standard used.

Measure the absorbances at 360 nm wavelength against the blank tube with methanol in the 1 cm cuvette. Plot the absorbances against the concentrations and fit a linear equation to the curve.

G.4.2 Sample preparation

Use the following procedure:

a) The samples should be extracted in accordance with Annex A. Prepare a working solution by pipetting 1,5 ml of propolis extract solution, S_V, (0,5 ml of each of triplicate extract solution) to a 10 ml volumetric flask and fill to the mark with ethanol 80 %.

b) The procedure given in steps c) to k) is carried out in triplicate.

c) Weigh 1,0 g (with an accuracy of 0,000 1 g) polyamide powder onto the glass evaporating dish. Pipette 1,0 ml, V_1, of the sample solution on the polyamide powder.

d) Stir evenly with a glass rod, then remove ethanol by evaporation on a water bath at 80 °C, electric heating plate or oven, and evaporate to remove the alcohol.

e) Transfer all sample-powder mix with toluene (approximately 20 ml to 30 ml) into a glass chromatographic column, put a little absorbent cotton above the sample-powder layer, open the valve and discard the toluene liquid.

f) When the toluene surface is about 0,5 cm away from the absorbent cotton layer, add 10 ml methanol, and control the dropping speed of 30 to 60 drops/min with the valve.

g) When the boundary line between toluene and methanol moves to the sand core layer at the bottom of the column, use a 50 ml measuring flask to receive the eluted solution.

h) When the liquid level of methanol drops to about 0,5 cm above the absorbent cotton layer at the top of the column, continue adding 10 ml of methanol and repeat four times. Close the valve, remove the measuring flask, and fill to the mark with methanol. Cap and mix the flask thoroughly by inversion several times.

i) Measure the eluted solution's absorbance at a wavelength of 360 nm against the blank tube with methanol in the 1 cm cuvette. Rutin is used as the standard substance and the standard curve method was used for quantitative determination.

j) Validation of elution effect: After removal of the receiving flask, continue to collect 5 ml of eluent in a separate container, and measure the absorbance of 360 nm. The absorbance value should be less than 10 % of the sample absorbance, indicating that the total flavonoids have been completely eluted. If the absorbance is greater than 10 %, repeat steps G.4.2.b to G.4.2.j with a smaller volume of propolis extract solution, S_V.

k) For each batch of samples, run a blank column using 1,0 ml to 2,0 ml of 80 % ethanol solution instead of a sample.

G.5 Calculation

Total flavonoid content in the raw propolis, F_2, expressed as a percentage of mass (calculated as rutin), is given by Formula (G.1):

$$F_2 = \frac{A}{m_1 \times S_v \times 20} \times 100 \quad \text{(G.1)}$$

where

A is the amount of flavonoids in the tested solution calculated from the calibration curve, in µg/ml;

m_1 is the mass value of raw propolis (the mean mass of raw propolis used in the extraction of Annex A), in g;

S_v is the volume of the propolis extract solution (according to Annex A) used to prepare the working solution (4,5 ml or other, according to the propolis type and quality)

G.6 Precision

The relative deviation of parallel experiments shall not be more than 10 %.

Annex H
(normative)

Chemical characterization of polyphenols in poplar propolis — HPLC/MS

H.1 Principle

Polyphenols, present as flavonoids and phenolic acids, in free and glycosylated forms, are extracted using ethanol 80 % as extractor solvent, according to Annex A, and the components are separated by HPLC and identified by mass spectrometry (MS) detection.

H.2 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

H.2.1 Formic acid 0,1 % volume/volume in water – HPLC mobile phase (A).

H.2.2 Acetonitrile MS-grade – HPLC mobile phase (B).

H.2.3 Water, chromatographic grade.

H.2.4 Ethanol /water solution 80/20 (volume/volume).

H.2.5 Calibration standards.

H.2.5.1 Apigenin (4′,5,7-trihydroxyflavone) standard: purity = 98 %, CAS Registry Number®[1)]: 520-36-5.

H.2.5.2 Caffeic acid (3,4-Dihydroxybenzeneacrylic acid) standard: purity = 98 %, CAS: 331-39-5.

H.2.5.3 CAPE (caffeic acid phenethyl ester) standard: purity ≥ 97 %, CAS: 104594-70-9.

H.2.5.4 p-coumaric acid ((2E)-3-(4-Hydroxyphenyl)prop-2-enoic acid) standard: purity = 98 %, CAS: 501-98-4.

H.2.5.5 Chrysin (5,7-Dihydroxy-2-phenyl-4H-chromen-4-one) standard: purity ≥ 99 %, CAS: 480-40-0.

H.2.5.6 Ferulic acid ((2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) standard: purity ≥ 99 %, CAS: 1135-24-6.

H.2.5.7 Galangin (3,5,7-Trihydroxy-2-phenylchromen-4-one) standard: purity ≥ 97 %, CAS: 548-83-4.

1) CAS Registry Number® is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

H.2.5.8 Pinobanksin ((2S,3R)-3,5,7-Trihydroxy-2-phenyl-chromen-4-one) standard: purity > 95 %, CAS: 548-82-3.

H.2.5.9 Pinocembrin ((2S)-5,7-dihydroxy-2-phenyl-2,3-dihydrochromen-4-one) standard: purity ≥ 95 %, CAS: 480-39-7.

H.2.6 Individual polyphenol stock solutions A – 1 000 µg/ml.

Accurately weigh 10 mg of each dry standard into a separate calibrated 10 ml brown glass volumetric flask. Record the actual mass. Add a small amount of 80/20 (volume/volume) ethanol/water (H.2.4) to each flask and swirl to dissolves the contents, before making up to the 10 ml mark with 80/20 (volume/volume) ethanol/water (H.2.4) and mix thoroughly. The stock solutions are stable for six months at –20 °C.

H.2.7 Mixed polyphenol stock solution B – 100 µg/ml.

Add the volumes of each individual polyphenol stock solution A given in Table H.1 to a calibrated brown glass 10 ml volumetric flask and fill to the mark with 80/20 (volume/volume) ethanol/water (H.2.4). The stock solution is stable for six months at –20 °C.

Table H.1

Standard at final concentration of 100 µg/ml	Volume of 1 000 µg/ml individual stock solution A (ml)
Apigenin	1,00
Caffeic acid	1,00
CAPE	1,00
p-coumaric acid	1,00
Chrysin	1,00
Ferulic acid	1,00
Galangin	1,00
Pinobanksin	1,00
Pinocembrin	1,00

H.2.8 Polyphenol mix standard working solution, linear range 10 µg/ml to 100 µg/ml.

Dilute the mixed polyphenol stock solution B – 100 µg/ml as shown in Table H.2.

Table H.2

Concentration of polyphenol mix standard working solution µg/ml	Volume mixed polyphenol stock solution B-100 µg/ml ml	Volume of 80/20 (volume/volume) ethanol/water (H.2.4) ml
10	0,10	0,90
25	0,25	0,75
50	0,50	0,50
75	0,75	0,25
100	1,00	0,00

The concentrations of the calibration curve are a suggestion, in the specified range. It is necessary to have at least five points to meet a good calibration curve. These standards should be prepared fresh each day as required.

H.3 Apparatus and equipment

H.3.1 Usual laboratory glassware.

H.3.2 Analytical balance suitable to perform weighing to an accuracy of within 0,000 1 g.

H.3.3 100 µl, 1 000 µl and 5 000 µl manual/automatic/electronic pipette.

H.3.4 10 ml test tube with screw cap.

H.3.5 5 ml plastic syringe.

H.3.6 Polyvinylidene fluoride (PVDF) syringe filters, 0,45 µm, 13 mm.

H.3.7 10 ml brown glass volumetric flasks.

H.3.8 Analytical system, comprising a HPLC ternary pump equipped with reverse phase chromatographic column (internal diameter 4,6 mm, length 25 cm, size 5 µm, 80 A°, type Discovery-C18[2)] with a mass (MS) detector type single quadrupole from Waters[3)] and an integration system. The use of MS spectra for peak identification.

H.4 Procedure

H.4.1 Sample preparation

The samples should be extracted in accordance with Annex A. Prepare a working solution by pipetting 1,5 ml of propolis extract solution, S_V, (0,5 ml of each triplicate extract solution) to a calibrated 10 ml volumetric flask, *V*, and fill to the mark with 80/20 (volume/volume) ethanol/water mix. Pass the solution through a PVDF membrane syringe filter.

H.4.2 HPLC analysis

H.4.2.1 General

Inject 10 µl of sample onto the HPLC column. The first sample injected in a series of analysis shall be a blank consisting of an ethanol/water solution 80/20 (volume/volume). There shall be no interfering signals present during the chromatographic run (n = 3).

H.4.2.2 MS detector parameters

Set the MS detector in negative ionization mode. Set the heat source to 600 °C. Scan spectra from 50 Da to 1 500 Da (2 to 10 full scans/s).

H.4.2.3 HPLC conditions

The example operating conditions for a Discovery-C18[2)] column are reported as shown in Table H.3 in the scheme.

2) The Discovery-C18 column is an example of a suitable commercially available chromatographic column. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

3) The detector from Waters is an example of a suitable commercially available MS instrument. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

Table H.3

Time min	**Flow** ml/min	**A** %	**B** %
0	1,00	80	20
6	1,00	80	20
10	1,00	70	30
40	1,00	60	40
80	1,00	10	90
90	1,00	10	90
90,1	1,00	80	20
100	1,00	80	20

The binary gradient is programmed to ensure that no molecular species remain in the column and that the elution from column is complete. The solvents for the elution shall first be degassed (for models of HPLC that have automatic degassing systems, solvent pre-degassing is not always required).

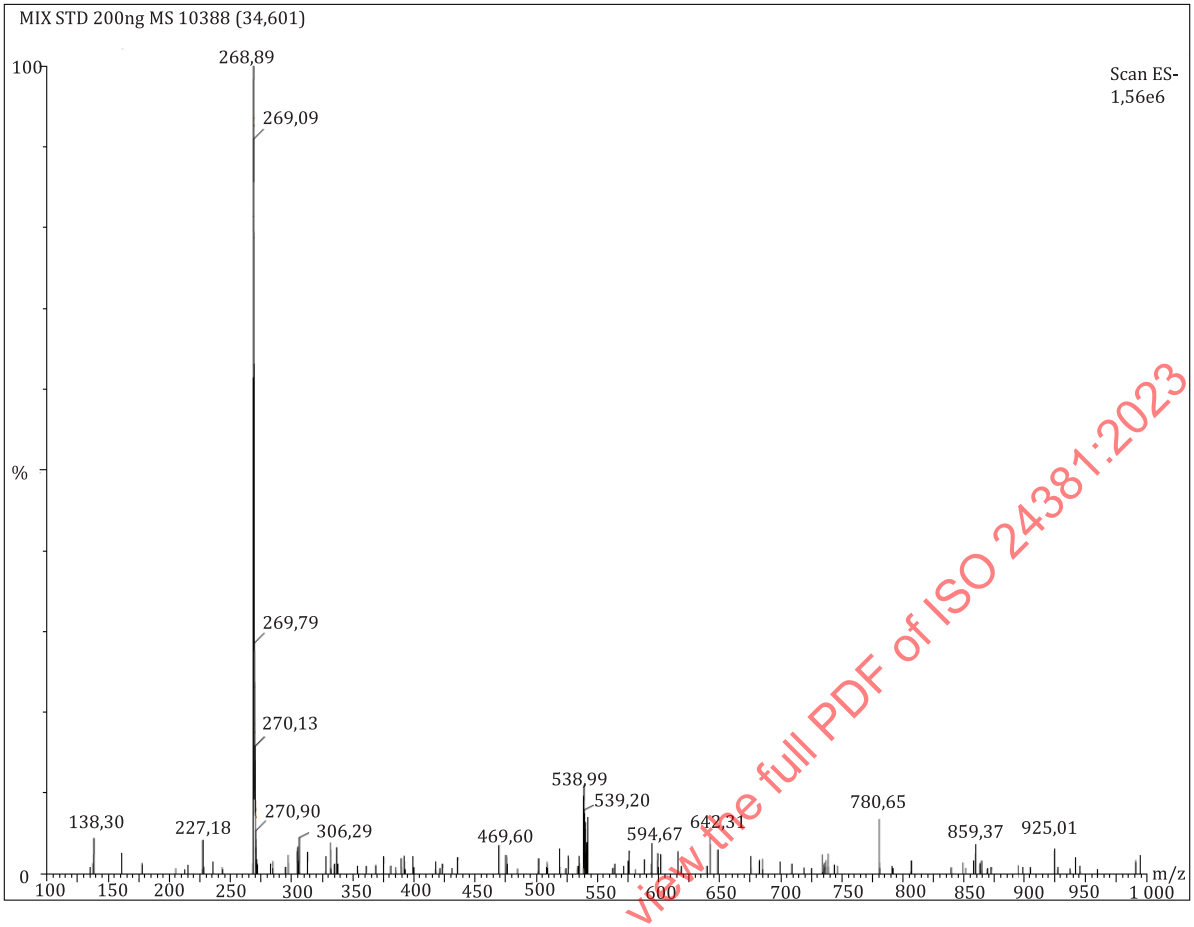
The HPLC column shall be conditioned for at least 20 min with the initial gradient T = 0. Initially, 10 µl of external standard solution is injected and once the run is completed, 10 µl of the extracted sample solution is injected (n = 3) (according to the sample preparation presented in H.4.1).

At the end of the analyses, the system shall be conditioned with acetonitrile/water 50/50 (volume/volume) at a flow of 1 ml/min for at least 60 min. Store the washed chromatographic column in acetonitrile with the end fitting closed.

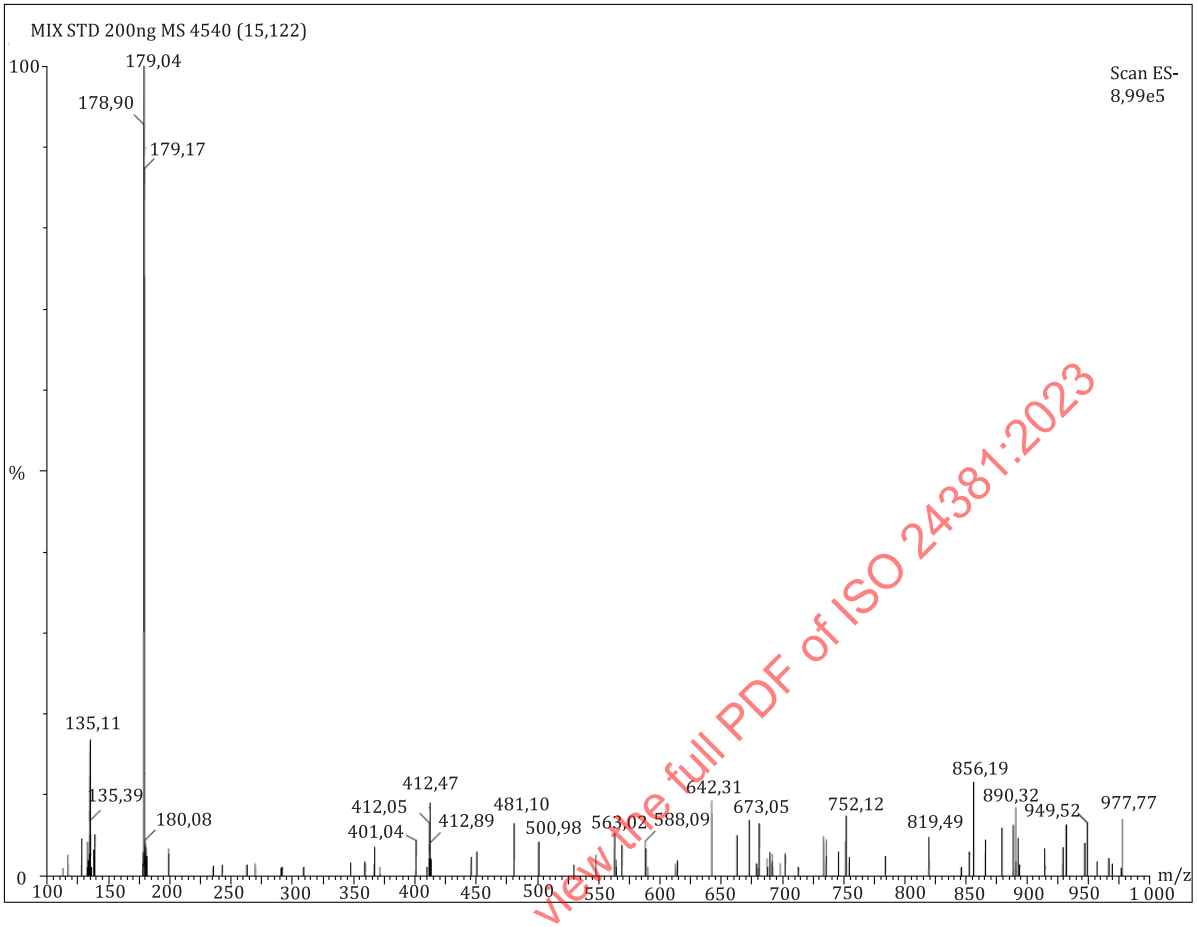
H.4.2.4 Peaks identification

Peaks identification is carried out from MS detector and from the retention time by comparison with the external reference standard solutions. The substances requested are apigenin, caffeic acid, CAPE, p-coumaric acid, chrysin, ferulic acid, galangin, pinobanksin and pinocembrin, see Figure H.1.

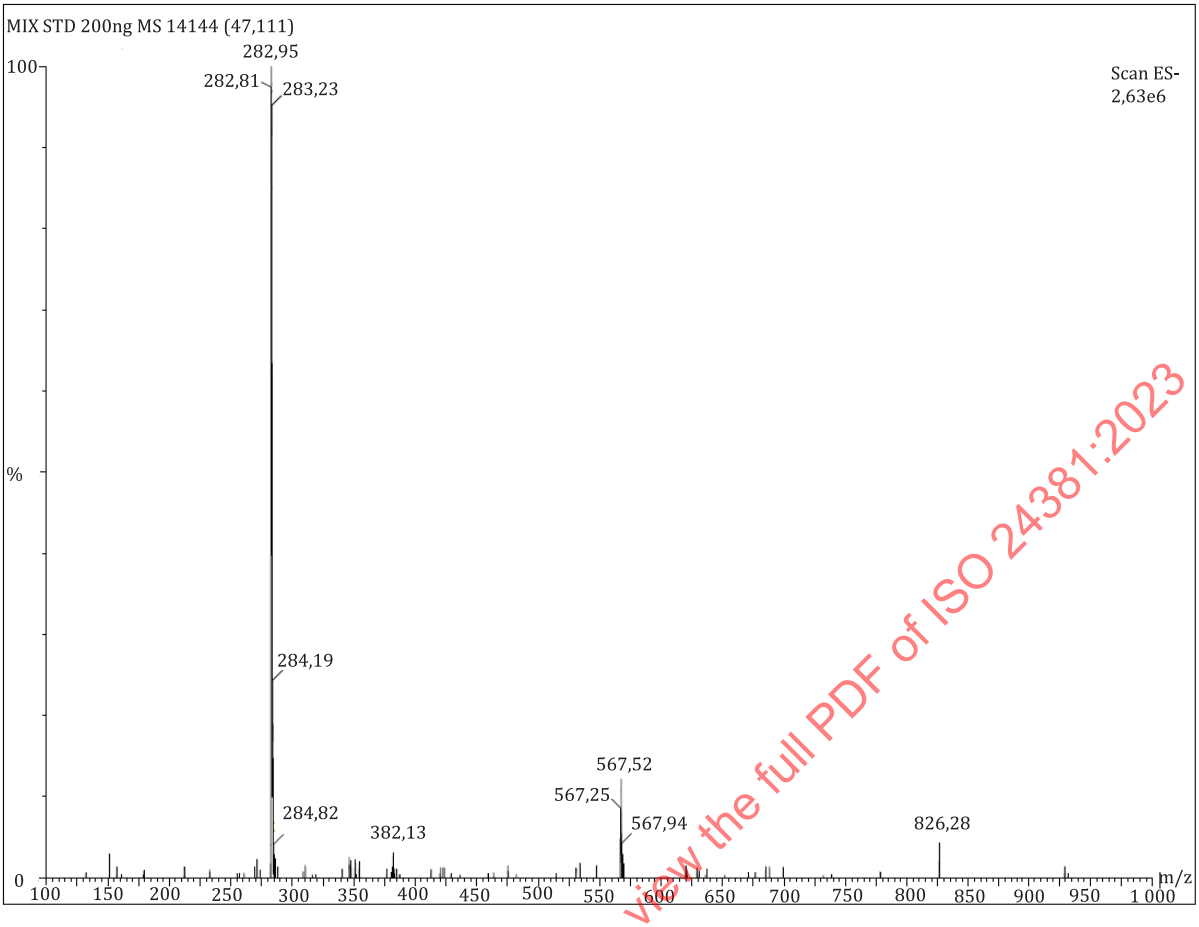
Mass spectra of selected reference standard useful for their identification are illustrated in the Figure H.1.



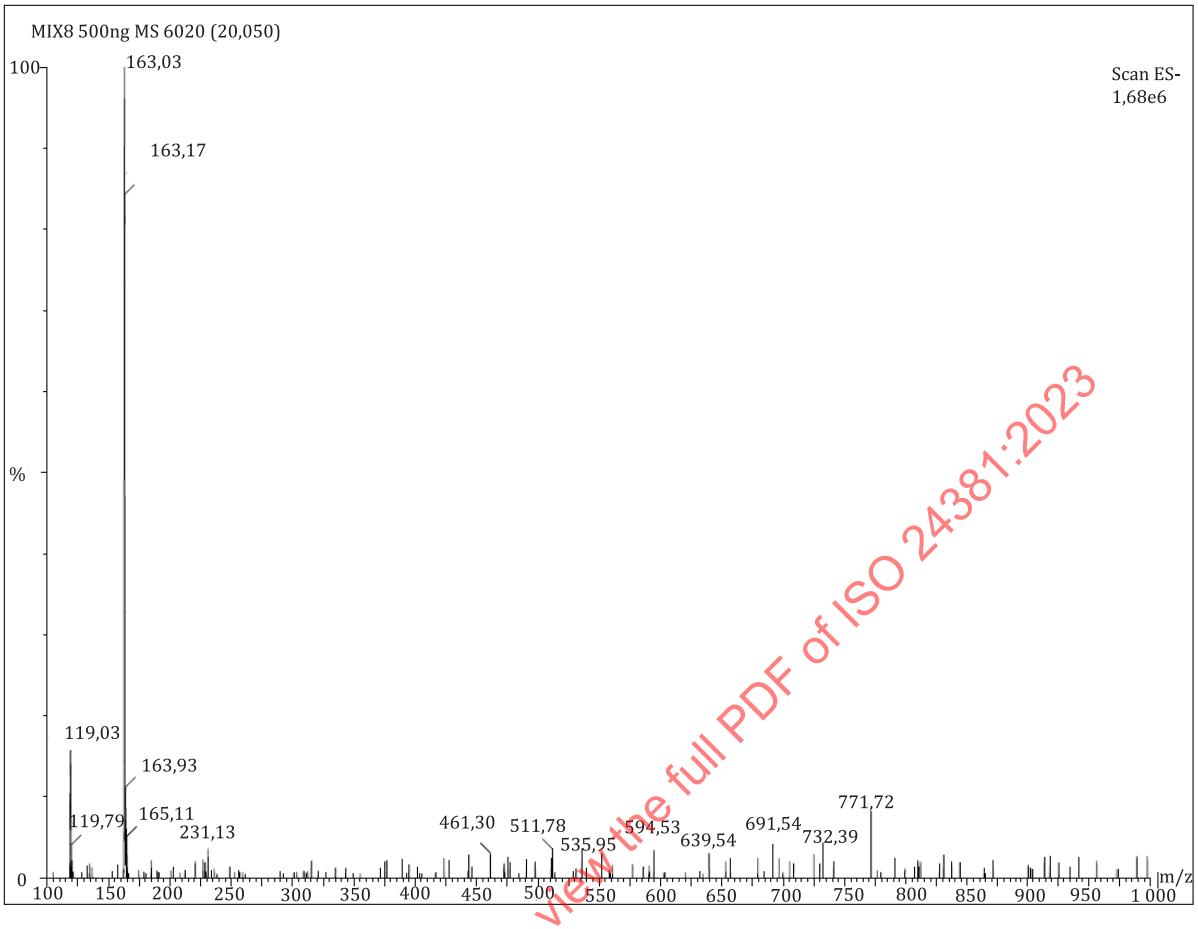
a) Apigenin



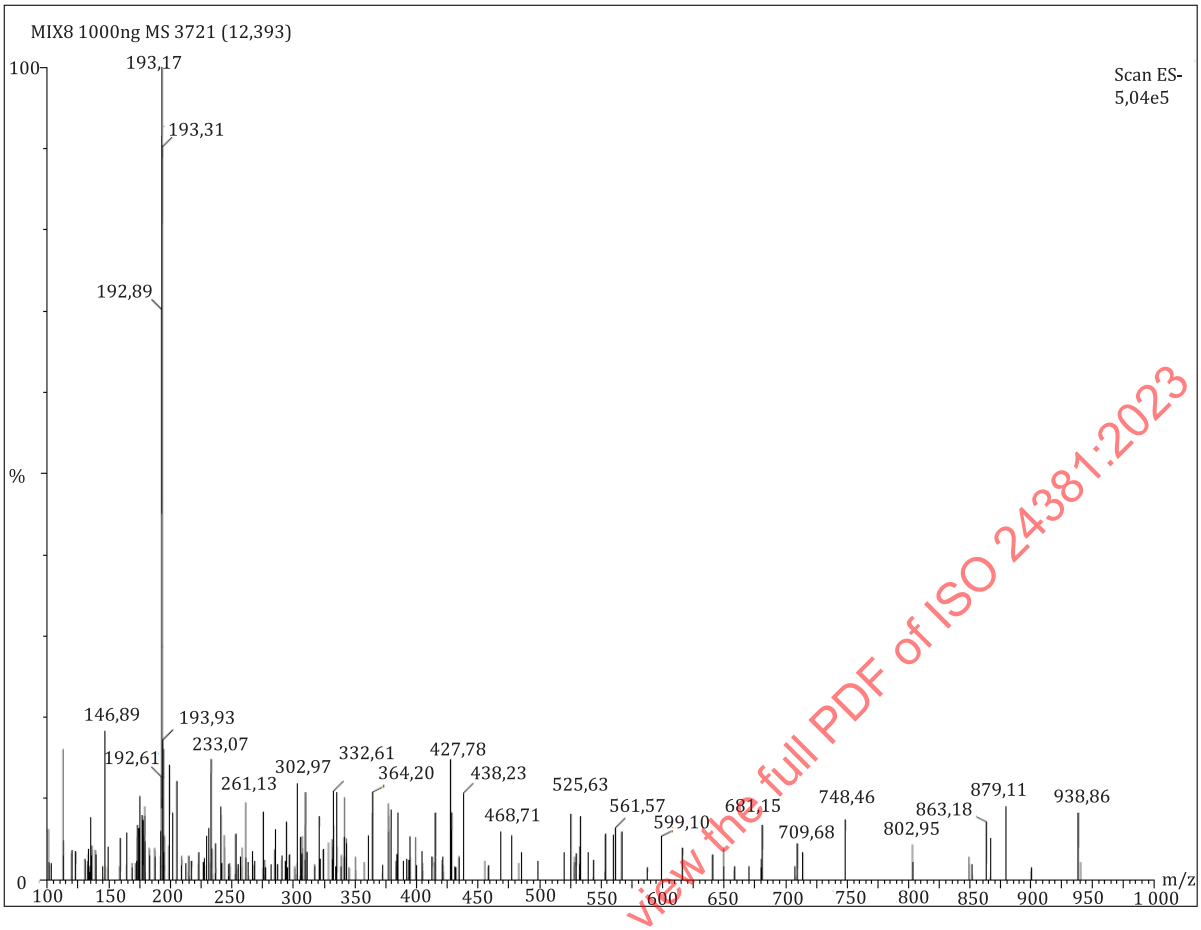
b) Caffeic acid



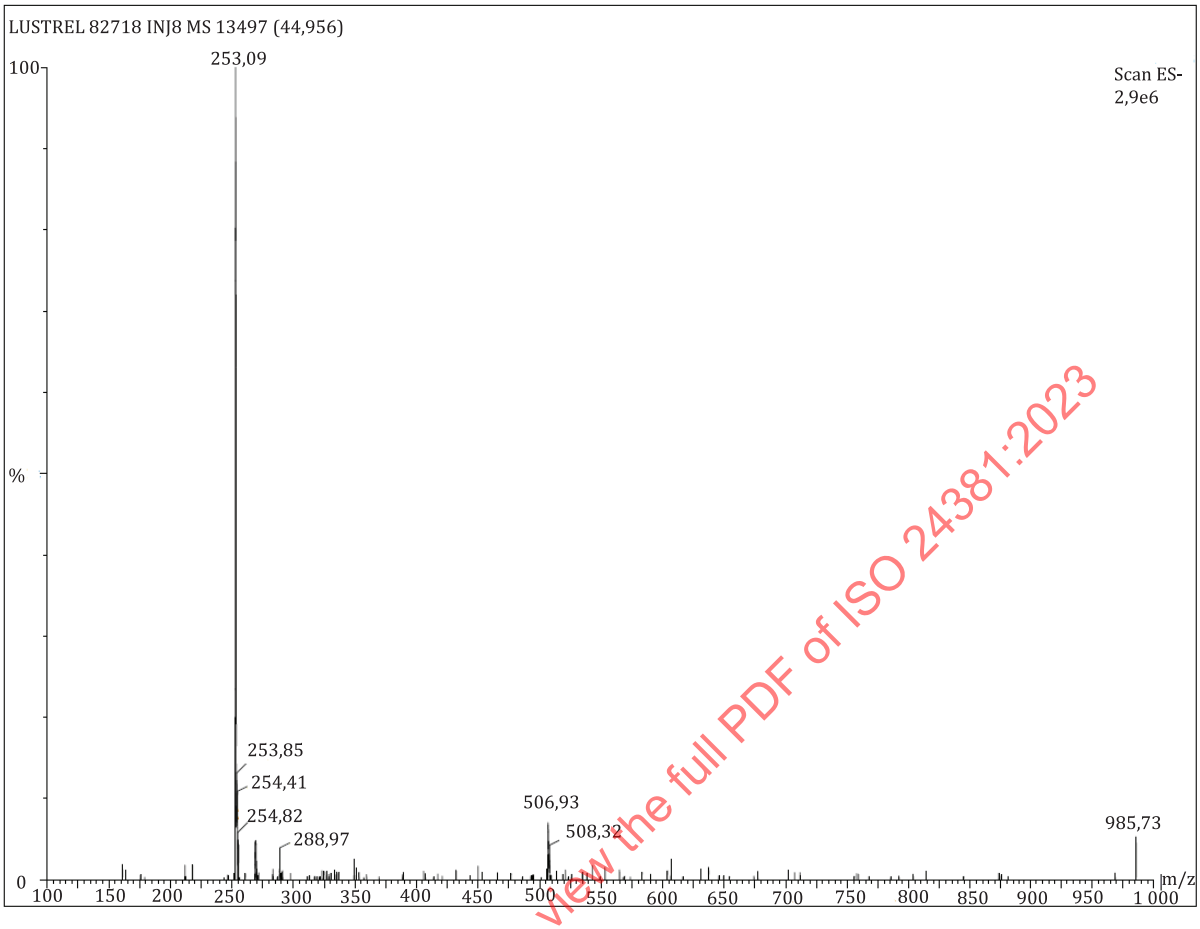
c) CAPE



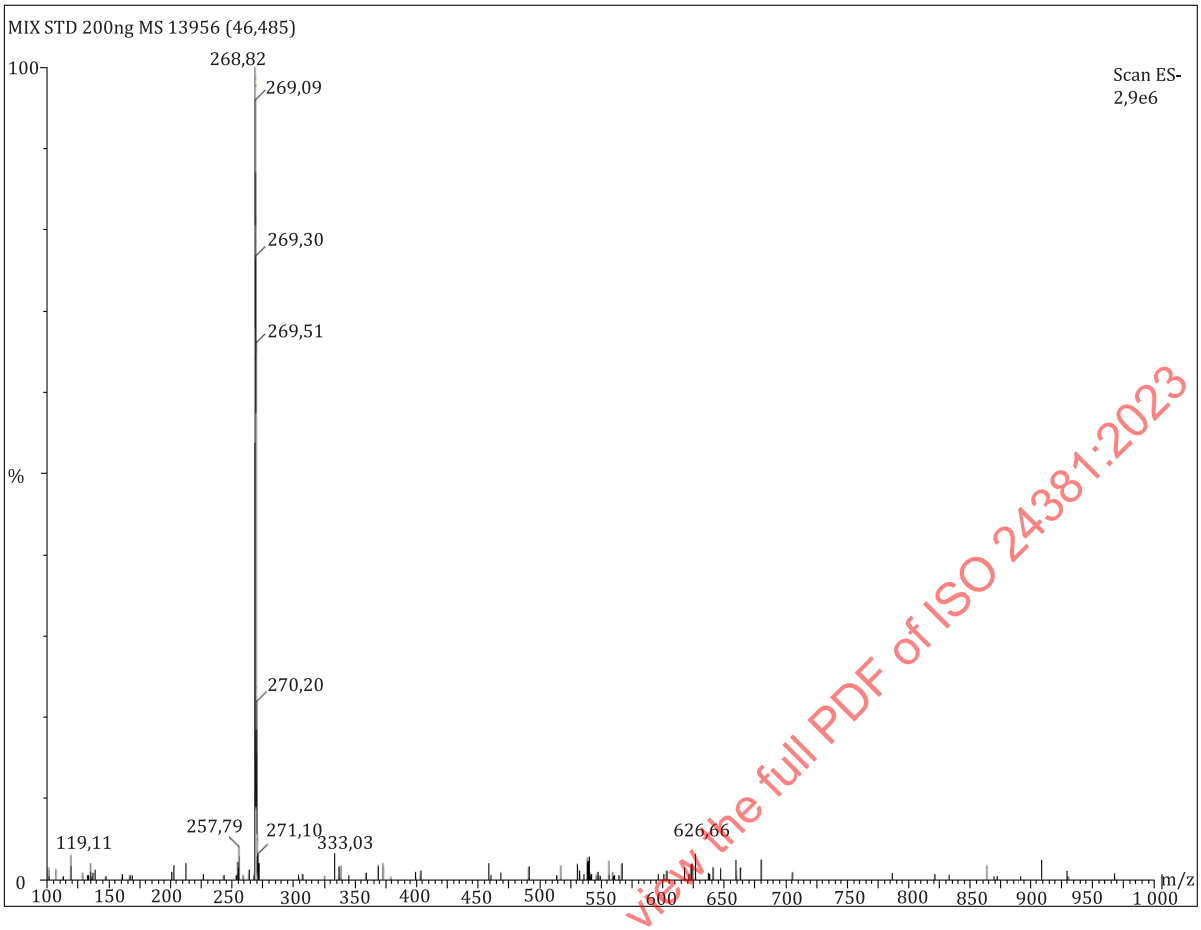
d) *p*-Coumaric acid



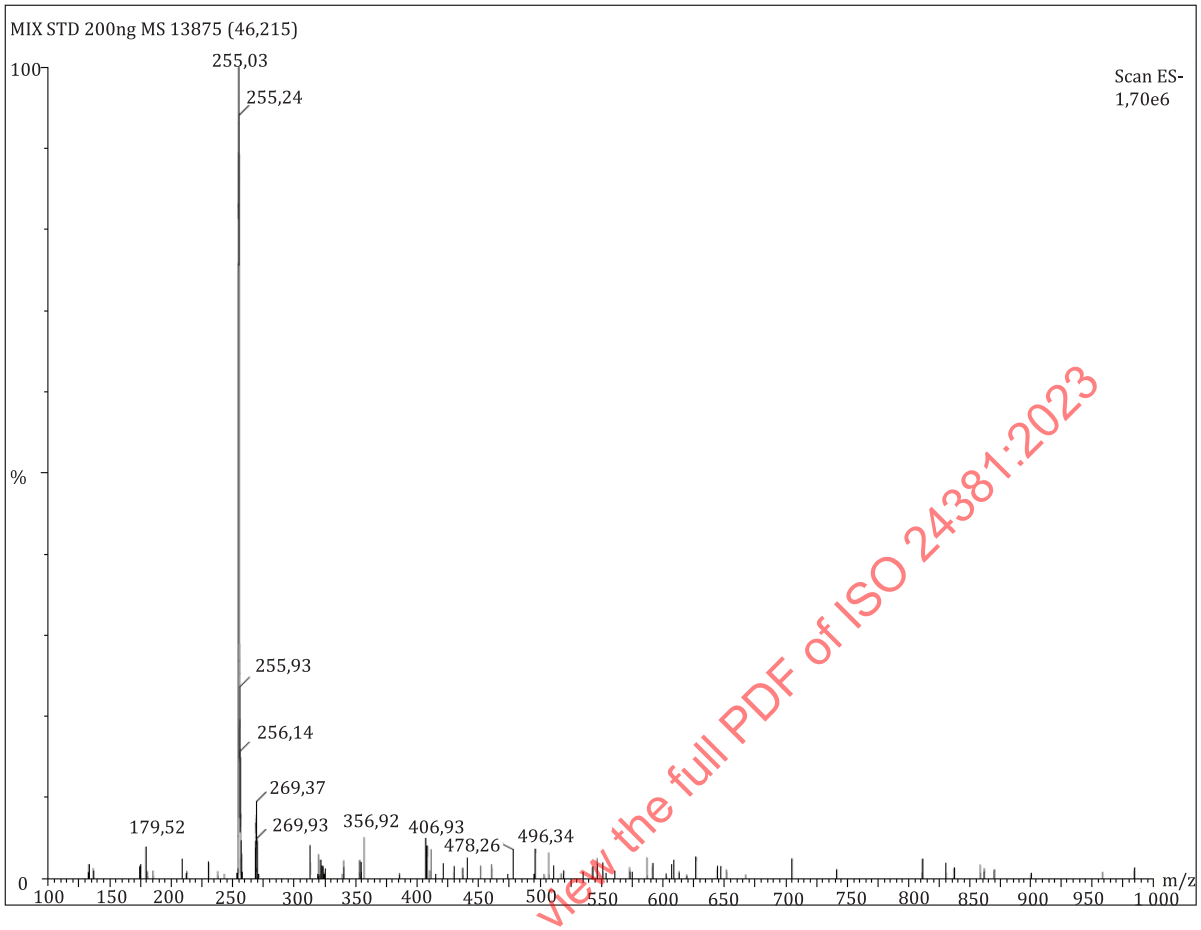
e) Ferulic acid



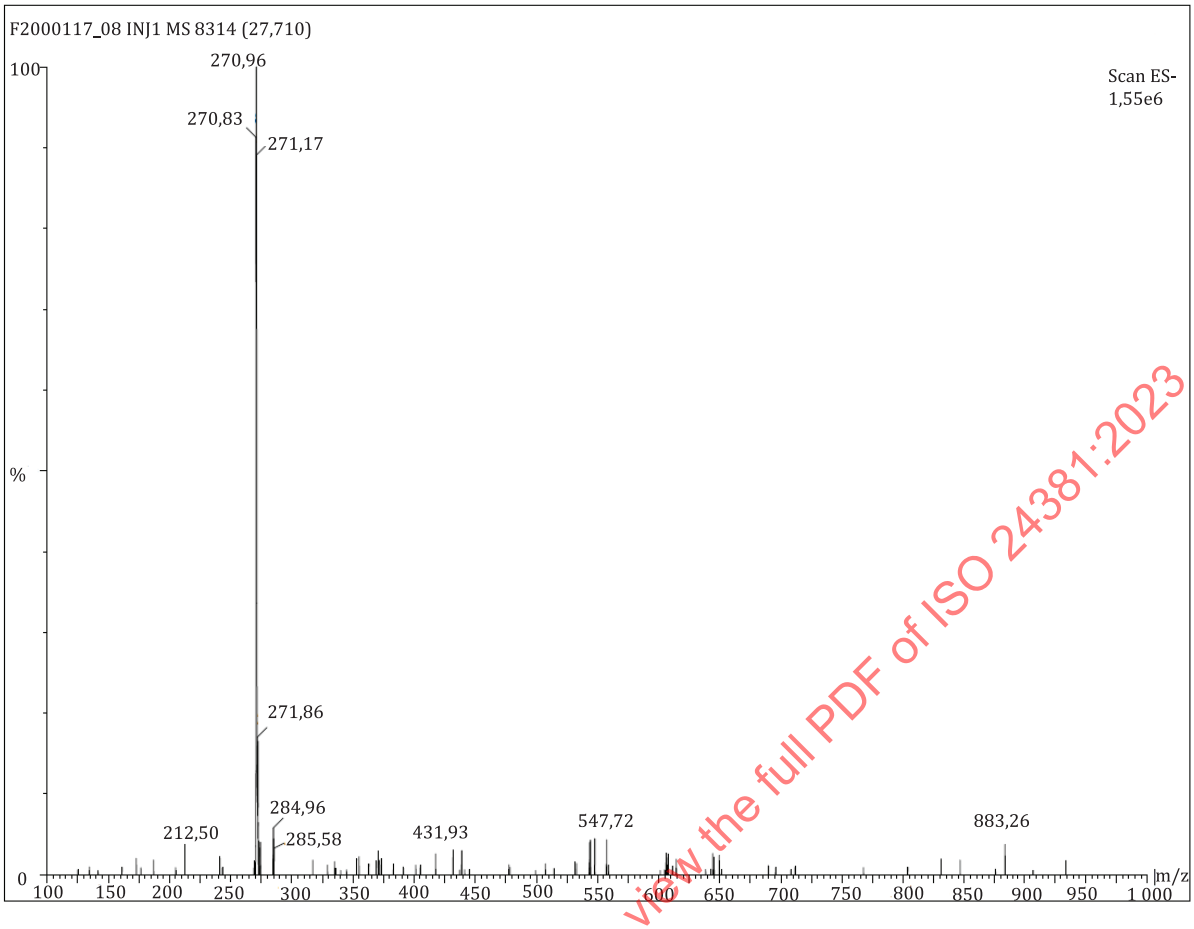
f) Chrysin



g) Galangin



h) Pinocembrin

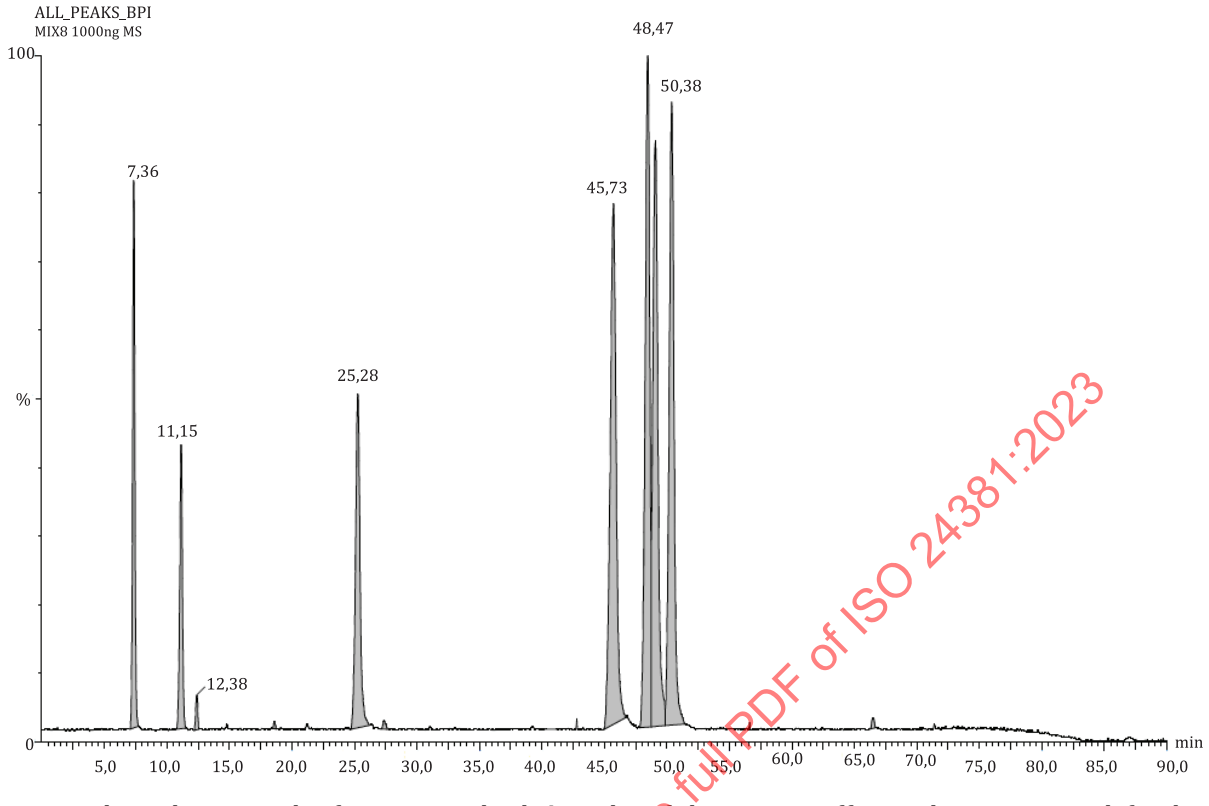


i) Pinobanksin

Figure H.1 — Peaks identification

H.4.2.5 Chromatograms

A typical chromatogram of the eight external reference standards is illustrated in Figure H.2



NOTE The eight external reference standards in order of elution are caffeic acid, coumaric acid, ferulic acid, apigenin, chrysin, pinocembrin, galangin and CAPE.

Figure H.2 — Typical chromatogram of the eight external reference standards

Annex I
(normative)

Chemical characterization of polyphenols in green propolis — HPLC/PDA

I.1 Principle

The samples are extracted using ethanol 80 % as extractor solvent, according to Annex A, and then detected by a photodiode array detector (PDA) at 280 nm and 340 nm by reversed phase high performance liquid chromatography (RP-HPLC), and quantified by external standard method. Qualitative and quantitative analysis is based on retention time, peak area and spectral profile.

I.2 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

I.2.1 Methanol chromatographic grade – HPLC mobile phase (B).

I.2.2 Formic acid 0,1 % volume/volume in water – HPLC mobile phase (A).

I.2.3 Ethanol solution 80 % (volume/volume).

I.2.4 Water-chromatographic grade.

I.2.5 Filter membrane: 0,45 µm organic cellulose filter membrane.

I.2.6 Calibration standards.

I.2.6.1 Caffeic acid (3,4-Dihydroxycinnamic acid) standard: purity ≥ 98 %, CAS: 331-39-5.

I.2.6.2 p-Coumaric acid (trans-4-Hydroxycinnamic acid) standard: purity ≥ 98 %, CAS: 501-98-4.

I.2.6.3 Artepillin C (3,5-diprenyl-4-hidroxycinnamic acid) standard: purity ≥ 98 %, CAS: 72944-19-5.

I.2.6.4 Baccharin (10-diepoxy-9,10-dihydro-4′-hydroxy-7′-(1-hydroxyethyl)) standard: purity ≥ 98 %, CAS: 61251-97-6.

I.2.7 Individual polyphenol stock solutions A – 1 000 µg/ml.

Prepare an individual stock solution for each polyphenol by weighing 10 mg of standard in a 10 ml brown glass volumetric flask. Record the actual mass. Add a small amount of ethanol solution 80 % (volume/volume) (I.2.3) to each flask and swirl to dissolves the contents, before making up to the 10 ml mark with the same solvent and mixing thoroughly. The stock solutions A is stable for six months at –20 °C.

I.2.8 Mixed polyphenol stock solution B – 100 µg/ml.

Add the volumes of each individual polyphenol stock solution A given in Table I.1 to a calibrated brown glass 10 ml volumetric flask and fill to the mark with ethanol solution 80 % (volume/volume) (I.2.3). The stock solution is stable for six months at –20 °C.

Table I.1

Standard at final concentration of 100 µg/ml	Volume of 1 000 µg/ml individual stock solution A (ml)
Caffeic acid	1,00
p-coumaric acid	1,00
Artepillin C	1,00
Baccharin	1,00

I.2.9 Polyphenol mix standard working solution, linear range 10 µg/ml to 100 µg/ml.

Dilute the mixed polyphenol stock solution B – 100 µg/ml as shown in Table I.2. Alternative volumes can be prepared as long as the same ratios of stock standard and ethanol solution 80 % (volume/volume) are used.

Table I.2

Concentrations of polyphenol mix standard working solution µg/ml	Volume mixed polyphenol stock solution B – 100 µg/ml ml	Volume of ethanol solution 80 % (volume/volume) ml
10	0,10	0,90
25	0,25	0,75
50	0,50	0,50
75	0,75	0,25
100	1,00	0,00

The concentrations of the calibration curve are a suggestion, in the specified range. It is necessary to have at least five points to meet a good calibration curve. These standards should be prepared fresh each day as required.

I.3 Apparatus and equipment

I.3.1 Usual laboratory glassware.

I.3.2 Analytical balance, suitable to perform weighing to an accuracy of within 0,000 1 g.

I.3.3 Manual pipette or electronic pipette, 100 µl and 1 000 µl.

I.3.4 Organic cellulose syringe filters, 0,45 µm, 15 mm.

I.3.5 Calibrated 10 ml brown glass volumetric flasks.

I.3.6 Analytical system, comprising a HPLC quaternary pump equipped with reverse phase chromatographic column (internal diameter 4,6 mm, length 25 cm, size 5 µm, type Shim-pack C-18[4]) with a PDA from Shimadzu[5] and integration system. The use of spectral profile for peak identification.

I.4 Procedure

I.4.1 Sample preparation

The samples should be extracted in accordance with Annex A. Prepare a working solution by pipetting 1,5 ml of propolis extract solution, S_V, (0,5 ml of each triplicate extract solution) to a calibrated 10 ml volumetric flask, *V*, and fill to the mark with ethanol solution 80 % (volume/volume).

The sample solution should be filtered by 0,45 µm filter membrane and the filtrate was used for liquid chromatographic determination.

I.4.2 HPLC analysis

I.4.2.1 General

Inject 10 µl of polyphenol mix standard working solution and sample solution (n = 3) into the HPLC column according to the following (I.4.2.2, I.4.2.3) chromatographic analysis conditions. Inject a blank sample (I.2.3) under the same conditions. The results of blank test shall not interfere with the measurement of propolis components in the chromatogram.

I.4.2.2 PDA detector and column temperature parameters

Set the detection wavelength to 280 nm and 340 nm and column temperature from 25 °C to 40 °C.

I.4.2.3 HPLC conditions

The example operating conditions for a Shim-pack-C18[4] column are reported as shown in Table I.3 in the Scheme:

Table I.3

Time min	**Flow** ml/min	**A** %	**B** %
0	0,8	80	20
70	0,8	5	95
75	0,8	80	20
77	0,8	80	20

The binary gradient is programmed to ensure that no molecular species remain in the column and that the elution from column is complete. The solvents for the elution shall first be degassed (for models of HPLC that have automatic degassing systems, solvent pre-degassing is not always required).

The HPLC column shall be conditioned for at least 20 min with the initial gradient T = 0.

At the end of the analyses, the system shall be conditioned with methanol at a flow of 1 ml/min for at least 60 min. Store the washed chromatographic column in methanol with the end fitting closed.

4) The Shim-pack-C18 column is an example of a suitable commercially available chromatographic column. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

5) The detector from Shimadzu is an example of a suitable commercially available PDA instrument. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.