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Spices — Saffron (*Crocus sativus* L.) —

Part 2: **Test methods**

*Épices — Safran (*Crocus sativus *L.) —*

Partie 2: Méthodes d'essai

Reference number
ISO 3632-2:2010(E)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 3632-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 7, *Spices, culinary herbs and condiments*.

This second edition of ISO 3632-2 cancels and replaces ISO/TS 3632-2:2003, which has been technically revised.

ISO 3632 consists of the following parts, under the general title *Spices — Saffron* (Crocus sativus *L.*):

— *Part 1: Specification*

— *Part 2: Test methods*

Spices — Saffron (*Crocus sativus* L.) —

Part 2:
Test methods

1 Scope

This part of ISO 3632 specifies test methods for dried saffron obtained from the *Crocus sativus* L. flower.

It is applicable to saffron:

a) filaments and cut filaments;

b) powder.

2 Normative references

The following referenced documents are indispensable for the application 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 928, *Spices and condiments — Determination of total ash*

ISO 930, *Spices and condiments — Determination of acid-insoluble ash*

ISO 941, *Spices and condiments — Determination of cold water-soluble extract*

ISO 948, *Spices and condiments — Sampling*

ISO 3632-1, *Spices — Saffron (*Crocus sativus *L.) — Part 1: Specification*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 3632-1 and the following apply.

3.1
moisture and volatile matter content
loss of mass fraction determined under the conditions specified in this part of ISO 3632

NOTE Moisture and volatile matter content is expressed as a percentage mass fraction of the sample.

3.2
colouring strength

$A^{1\%}_{1\,cm}$, 440 nm

absorbance at the maximum wavelength (about 440 nm) of crocins for a 1 g/100 ml solution of test sample using a 1 cm quartz cell

NOTE Colouring strength is mainly due to the content of crocins.

3.3
UV-vis profile of saffron extract
spectrum obtained between 200 nm and 700 nm

NOTE An example is given for information in Figure C.1.

3.4
limit of detection
LOD
minimum amount or concentration of the analyte in a test sample which can be detected reliably, but not necessarily quantified, as demonstrated by a collaborative trial or other appropriate validation

3.5
minimum required performance limit
MRPL
minimum content of an analyte in a sample, which at least has to be detected and confirmed

4 Tests and sample sizes

4.1 Minimum mass of the laboratory sample

Sampling methods for spices and condiments are specified in ISO 948.

Considering the high cost of saffron, the mass of sample received in the laboratories for carrying out the tests is often limited. The minimum mass of the laboratory sample shall be 23 g (11,5 g $\times$ 2) for saffron filaments and cut filaments and 13,5 g (6,75 g $\times$ 2) for saffron powder in order to carry out the standard analyses in duplicate.

It is recommended that larger quantities of sample be placed at the disposal of the laboratories in case of dispute.

Since the mass of the test portion is low, it is advisable that it be taken from a homogenized sample.

4.2 Tests required and test sample sizes

See Table 1 for saffron in filaments, cut filaments and Table 2 for saffron in powder.

Table 1 — Saffron in filaments and cut filaments forms

Analysis step	Procedure (laboratory sample: 11,5 g × 2 = 23 g)	Test sample g	Comments	Corresponding clause
1	Identification test	5	New test sample Non-destructive test	5
2	Microscopic examination	0,05	Test sample from step 1	6
3	Determination of floral waste content	3	Test sample from step 1 Non-destructive test	8
4	Determination of foreign matter	3	Test sample from step 3 Test sample is reconstituted after reincorporating floral waste	9
5	Determination of extract soluble in cold water	2	Test sample from step 4	11
6	Determination of moisture and volatile matter content	2,5	New test sample Keep the test sample for determination of total ash and acid-insoluble ash	7
7	Determination of total ash	2	Remains of the test sample from step 6	12
8	Determination of acid-insoluble ash	—	Test sample produced after step 7	13
9	Crushing and sieving	4	New test sample Carry out the sieving in accordance with Clause 10 to obtain a powder of which 95 % mass fraction passes through a 500 µm sieve. Reincorporate whatever remains on the sieve in the receptacle of the sieve	10
10	Determination of main characteristics	0,5	Test sample from step 9, after sieving	14
11	Thin-layer chromatography (TLC): identification of artificial colorants	0,5	Test sample from step 9, before sieving HPLC (step 12) may alternatively or additionally be performed. In the latter case, use the extract for both methods	15
12	High performance liquid chromatography (HPLC): identification of artificial colorants	0,5	Test sample from step 9, before sieving TLC (step 11) may alternatively or additionally be performed. In the latter case, use the extract for both methods	16
NOTE	There remain 4,50 g laboratory sample which can be used for further tests or to repeat certain analyses if necessary.			

Table 2 — Saffron in powder form

Analysis step	Procedure (laboratory sample: 6,75 g × 2 = 13,5 g)	Test sample g	Comments	Corresponding clause
1	Identification test	0,2	New test sample Do not continue with the analysis if the colorimetric analysis is not correct	5
2	Microscopic examination	0,05	New test sample	6
3	Determination of moisture and volatile matter content	2,5	New test sample Keep the test sample for determination of total ash and acid-insoluble ash	7
4	Determination of total ash	2	Remains of the test sample from step 3	12
5	Determination of acid-insoluble ash	—	Remains of the test sample from step 4	13
6	Crushing and sieving	4	New test sample Verify that 95 % mass fraction of the powder passes through a 500 µm sieve. Reincorporate whatever remains on the sieve in the receptacle of the sieve	10
7	Determination of extract soluble in cold water	2	Test sample from step 6	11
8	Determination of main characteristics	0,5	Test sample from step 6, after sieving	14
9	Thin-layer chromatography (TLC): identification of artificial colorants	0,5	Test sample from step 6, before sieving HPLC (10) may alternatively or additionally be performed. In the latter case, use the extract for both methods	15
10	High performance liquid chromatography (HPLC): identification of artificial colorants	0,5	Test sample from step 6, before sieving TLC (9) may alternatively or additionally be performed. In the latter case, use the extract for both methods	16
NOTE	There remain 1 g laboratory sample which can be used for further tests or to repeat certain analyses if necessary.			

5 Identification test

5.1 General

This preliminary test may make the subsequent analyses unnecessary if it shows that the saffron is not pure.

5.2 Saffron in filaments and cut filaments

5.2.1 Principle

The saffron is examined visually with a magnifying glass.

5.2.2 Apparatus

5.2.2.1 **Magnifying glass**, with a magnification of 10 times max.

5.2.2.2 **Watch glass**, of suitable size.

5.2.3 Procedure

Spread out the test sample of saffron in filaments and cut filaments (Table 1) on the watch glass (5.2.2.2) and examine it with the magnifying glass (5.2.2.1).

5.2.4 Interpretation of results

All the filaments shall belong to the plant *Crocus sativus* L.

Reject the sample if vegetable matter other than that belonging to *Crocus sativus* L. is found.

5.3 Saffron in powder form

5.3.1 Principle

A colorimetric reaction is used.

5.3.2 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent quality.

5.3.2.1 **Sulfuric acid**, mass concentration 1,19 g/l.

5.3.2.2 **Diphenylamine solution**. Add 0,1 g diphenylamine to 20 ml sulfuric acid (5.3.2.1) and 4 ml water.

The diphenylamine shall not produce any coloured reaction with the sulfuric acid.

5.3.3 **Porcelain dish**, with flat bottom.

5.3.4 Procedure

Use the 0,2 g test sample of saffron (see Table 2) as test portion.

Gradually add the test portion to the porcelain dish (5.3.3) containing the diphenylamine solution (5.3.2.2).

5.3.5 Interpretation of results

Pure saffron immediately produces a blue colour, which rapidly turns reddish brown.

In the presence of nitrates, the blue colour persists.

6 Microscopic examination of saffron

6.1 General

The method is applicable to the examination of saffron in powder, filaments, and cut filament forms in order to determine whether the sample consists exclusively of stigmas belonging to *Crocus sativus* L. and to highlight any floral waste and foreign matter.

6.2 Principle

The identity of saffron in powder and crushed filament form is verified. Foreign matter and floral waste, if any, are identified by the observation of anatomical elements by using microscopy under the conditions described in 6.5. The percentages relating to the observed elements may be determined if required (see Annexes A and B).

6.3 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

6.3.1 Iodine in iodide solution, aqueous solution of iodine in potassium iodide.

In a 100 ml one-mark volumetric flask (6.4.5), equipped with a glass stopper, add 2 g iodine, 4 g potassium iodide, and about 10 ml water. Leave until completely dissolved, then make up to the mark with water. Stopper the flask.

6.3.2 Illuminating solution, either sodium hydroxide or potassium hydroxide at a mass concentration of 5 g/100 ml water or chloral hydrate with 80 g/100 ml water; dissolve when hot.

6.4 Apparatus

Usual laboratory equipment and in particular the following.

6.4.1 Slides.

6.4.2 Cover-glasses.

6.4.3 Scalpel.

6.4.4 Lancet needles.

6.4.5 One-mark volumetric flask, capacity 100 ml, ISO 1042[4] class A.

6.4.6 Syringe, graduated in microlitres, capable of delivering volumes of 50 µl.

6.4.7 Microscope, permitting observation with a magnification of 100 times and 400 times, optionally equipped with a device permitting observation in polarized light.

6.5 Procedure

6.5.1 Test portion

For each slide (6.5.2 to 6.5.4), take a test portion of the order of 0,001 g to 0,002 g saffron in powder (see 10.3) or crushed filament (see 10.2) form.

6.5.2 Preparation for observation in water

Prepare two slides as follows.

Deposit 50 µl of water on a slide. Using the tip of a scalpel or lancet needle, take the test portion (6.5.1), mix it with the water and wait for at least 5 min to ensure that the powder is adequately wet before covering with a cover slide.

6.5.3 Preparation for observation in an aqueous solution of sodium hydroxide, potassium hydroxide or chloral hydrate

Prepare two slides as indicated in 6.5.2, but replace water with the sodium hydroxide, potassium hydroxide or chloral hydrate aqueous solutions (6.3.2).

Wait for a few minutes for the medium to illuminate and observe for 10 min after adding the illuminating solution in order to avoid altering the cellular elements and ensuring they can be identified.

NOTE This observation enables illumination of the preparations by destroying totally or partially the major part of the cellular contents. The cellular elements are also made clearer and easier to observe, particularly the sclerous elements, vessels, fibres and epidermis.

6.5.4 Preparation for observation in aqueous iodine in iodide solution

Prepare a slide as indicated in 6.5.2, but replace the water with iodine in iodide solution (6.3.1).

NOTE This observation makes visible the starch grains which are stained blackish blue or blackish violet.

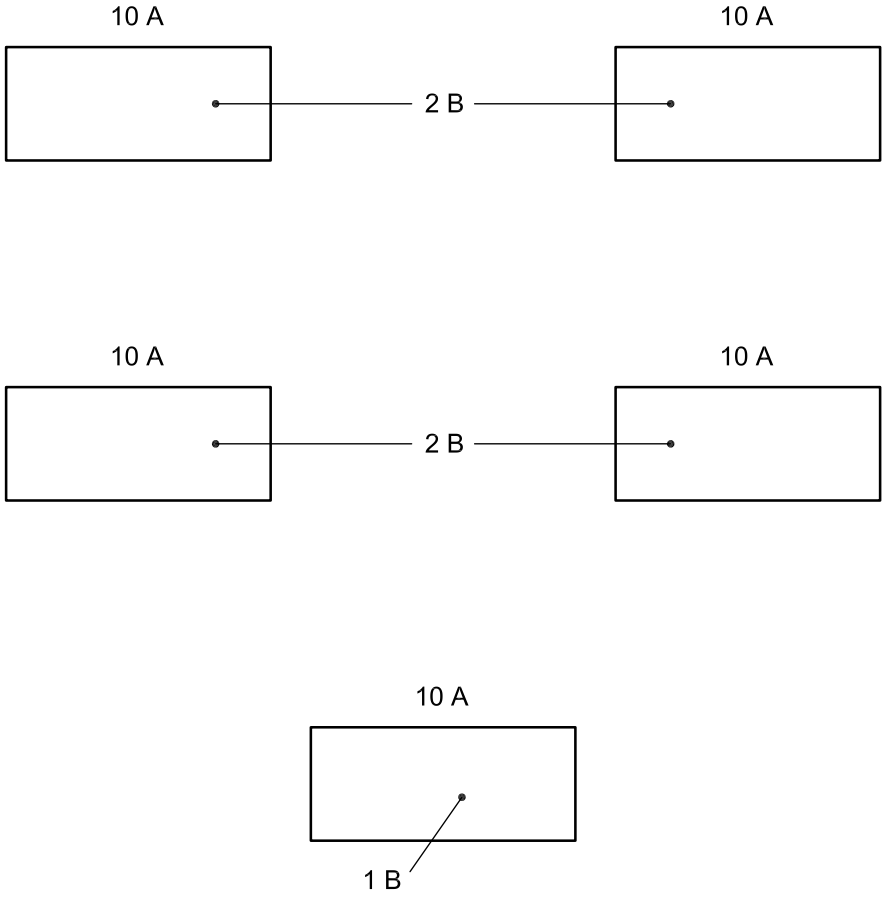
6.5.5 Observation, identification, and counting

Place each slide, prepared according to 6.5.2 to 6.5.4, under the microscope (6.4.7). Set the magnification at 100 times. Identify and count the elements observed with a magnification of 400 times (see 6.7).

NOTE The anatomical structures and exogenous elements are identified and counted for each slide on an observation of 10 fields.

If the microscope used (6.4.7) is equipped with a device permitting observation in polarized light, one of the two slides prepared in 6.5.2 should be so observed.

Figure 1 shows an example which summarizes all operations permitting counting.



Key

A field

B slide

Figure 1 — Example of counting procedure

6.6 Expression of results

An example is given for information in Annex A.

6.7 Microscopic examination

See reference photographs given for information in Annex B.

During the examination, the following elements can be observed:

a) fragments of the top extremity of the stigmas with large hair-like elongated papillae, after crushing the isolated papillae (Figure B.1);

b) epidermic debris of the stigmas which are characterized by small intussusceptions of the membrane (Figure B.2);

c) debris of the epidermis of the style, characterized by a sinuous wall (Figure B.3);

d) round pollen grains with a diameter of between 80 µm and 100 µm, with a smooth cell wall and finely granular exine (Figure B.4);

e) fragments of conductor elements made up of spiralled vessels (Figure B.5);

f) fragments of stamens (Figure B.6);

g) grains of starch (Figure B.7);

h) inorganic matter (Figure B.8);

i) fragments of straw (Figure B.9);

j) cells the content of which remain coloured despite the illuminating solution (Figure B.10).

6.8 Interpretation of microscopic observations

Evaluation of the relative percentage of each structure evaluated from the count table permits a check that the crushed saffron is mainly made up of fragments of stigmas to which fragments of styles and grains of pollen can be associated.

The observation should be reported in accordance with Tables A.1 and A.2.

NOTE The crushed saffron does not have sclerous cells, fibres, covert hair or starch grains. The contents of the cells dissolve in water to give an orange-yellow colour.

7 Determination of moisture and volatile matter content

7.1 General

This method is applicable to saffron in filaments, cut filaments and in powder forms.

NOTE The method of determination of the moisture content of spices and condiments described in ISO 939[3] is not applicable in the case of saffron because it requires the use of too large a test portion.

7.2 Principle

The sample is oven dried at 103 °C ± 2 °C for 16 h.

7.3 Apparatus

Usual laboratory apparatus and in particular the following.

7.3.1 Weighing dish or **evaporating dish**, provided with a lid or shoe glass.

7.3.2 Oven, capable of being maintained at 103 °C ± 2 °C.

7.3.3 Desiccator, containing an effective desiccant.

7.3.4 Analytical balance, capable of being read to the nearest 0,001 g.

7.4 Procedure

7.4.1 Test portion

7.4.1.1 Saffron in filaments and cut filaments

Weigh, to the nearest 0,001 g, approximately 2,5 g of sample (see Table 1) into the weighing dish or evaporating dish (7.3.1) previously dried and tared to the nearest 0,001 g.

7.4.1.2 Saffron in powder form

Weigh, to the nearest 0,001 g, approximately 2,5 g of sample (see Table 2) into the weighing dish or evaporating dish (7.3.1) previously dried and tared to the nearest 0,001 g.

7.4.2 Determination

Place the weighing dish or evaporating dish containing the test portion (7.4.1.1 or 7.4.1.2) uncovered in the oven (7.3.2) maintained at 103 °C and leave for 16 h. Cover with the lid or shoe glass, and allow it to cool in the desiccator (7.3.3). After cooling, weigh to the nearest 0,001 g.

Conserve the product for the later determination of total ashes (see Clause 12) and acid-insoluble ash (see Clause 13).

Carry out two determinations on the same laboratory sample.

7.5 Expression of results

The moisture and volatile matter content, w_{MV}, expressed as a percentage of the initial sample is equal to:

$$w_{MV} = (m_0 - m_4) \times \frac{100}{m_0} \%$$

where

m_0 is the mass, in grams, of the test portion;

m_4 is the mass, in grams, of the dry residue.

Take as the result, the arithmetic mean of the two determinations, if the repeatability conditions are met.

8 Determination of floral waste content of saffron in filaments and cut filaments

8.1 Principle

The floral waste present in a test portion is physically separated then weighed.

8.2 Apparatus

8.2.1 **Watch glass**.

8.2.2 **Small laboratory tongs**.

8.2.3 **Analytical balance**, capable of weighing to the nearest 0,01 g.

8.3 Procedure

8.3.1 Test portion

Weigh, to the nearest 0,01 g approximately 3 g of the test sample.

Since the mass of the test portion is low, it is advisable that it be taken from a homogenized sample.

8.3.2 Determination

Spread the test portion on a sheet of neutral grey paper. With the help of the small tongs (8.2.2), separate the different components of floral waste. Weigh on the analytical balance (8.2.3) to the nearest 0,01 g the previously dried watch glass (8.2.1).

Transfer the separated floral wastes to the shoe glass and weigh the whole to the nearest 0,01 g.

8.4 Expression of results

The floral waste content of the sample, w_F, expressed as a percentage by mass, is equal to:

$$w_F = (m_2 - m_1) \times \frac{100}{m_0} \%$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in grams, of the shoe glass;

m_2 is the mass, in grams, of the shoe glass containing the floral waste.

Take as the result, the arithmetic mean of the two determinations, if the repeatability conditions are met.

9 Determination of foreign matter content of saffron in filaments and cut filaments

9.1 Principle

The foreign matter present in a test portion is separated physically then weighed.

9.2 Apparatus

Use the same apparatus as specified in Clause 8.

9.3 Procedure

9.3.1 Test portion

Since the mass of the test portion is low, it is advisable that it be taken from a homogenized sample.

Reconstitute the test sample (approx. 3 g) by reincorporating the floral wastes previously separated and determined as in Clause 8, for saffron filaments and cut filaments. Homogenize well and then weigh the sample to the nearest 0,01 g.

9.3.2 Determination

Spread the test portion on a sheet of neutral grey paper. With the help of the small tongs, or with other appropriate means, separate the foreign matter from the test portion.

Weigh, to the nearest 0,01 g, the previously dried shoe glass.

Transfer the separated foreign matter to the shoe glass and weigh the whole to the nearest 0,01 g.

9.4 Expression of results

The foreign matter content of the sample, w_{FM}, expressed as a percentage by mass, is equal to:

$$w_{FM} = (m_3 - m_1) \times \frac{100}{m_0} \%$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in grams, of the shoe glass;

m_3 is the mass, in grams, of the watch glass containing the foreign matter.

10 Crushing and sieving of the samples for tests described in Clauses 6, 14, 15 and 16

10.1 Apparatus

10.1.1 Crusher, which shall:

a) be easy to dismantle and to clean, and have a minimum dead space;

b) permit a quick and uniform crushing without causing heat or loss of moisture;

c) avoid contact with the ambient air as much as possible;

d) permit a total recovery of all fragments of the sample;

e) not introduce any foreign substance into the sample.

10.1.2 Sieve, of 500 µm mesh, ISO 565[1].

10.2 Saffron in filaments and cut filaments

Crush the test sample (see Table 1) using the crusher (10.1.1) until 95 % mass fraction of the powder passes through the sieve (10.1.2).

Then, reincorporate the material remaining on the sieve and homogenize the whole.

10.3 Saffron in powder form

Sieve the test sample (see Table 2) using the sieve (10.1.2) in order to verify that 95 % mass fraction of the powder passes through it.

If this is not the case, crush the powder in the crusher (10.1.1) to obtain the required particle size.

Then, reincorporate the material remaining on the sieve and homogenize the whole.

11 Determination of extract soluble in cold water

Proceed in accordance with the method given in ISO 941.

For saffron in filaments, cut filaments and in powder forms, take a test portion of 2,00 g $\pm$ 0,01 g.

12 Determination of total ash

Proceed in accordance with the method given in ISO 928.

For saffron in filaments, cut filaments and in powder forms, use the sample which was used for the determination of the moisture content (7.4.2).

13 Determination of acid-insoluble ash

Proceed in accordance with the method given in ISO 930.

For saffron in filaments, cut filaments and in powder forms, use the sample which was used for the determination of the total ash (Clause 12).

14 Determination of the main characteristics using a UV-vis spectrometric method

14.1 General

This method enables the determination of the main characteristics of saffron connected with picrocrocin, safranal and crocin content. It is directly applicable to saffron in powder form provided that the powder conforms to the requirements of 10.3 and to saffron filaments and cut filaments after crushing and sieving in accordance with 10.2.

14.2 Principle

Measurement of the variation in optical density between 200 nm and 700 nm of an aqueous extract of saffron at ambient temperature.

14.3 Apparatus

Usual laboratory equipment and in particular the following.

14.3.1 Spectrometer, suitable for recording optical density in the ultraviolet-visible (UV-vis) band between 200 nm and 700 nm.

14.3.2 Quartz cell, with an optical pathlength of 1 cm.

14.3.3 One-mark volumetric flasks, capacity 200 ml and 1 000 ml, ISO 1042[4] class A, in anti-actinic glass.

14.3.4 One-mark pipette, capacity 20 ml, ISO 648[2] class A.

14.3.5 Filtration membrane, made of cellulose acetate or made of hydrophilic polytetrafluoroethylene (PTFE) [Millex-LCR[1)]] of porosity 0,45 µm.

14.4 Procedure

14.4.1 Test portion

Weigh, to the nearest milligram, 500 mg of the sample (see Table 1 or Table 2) in a shoe glass.

14.4.2 Determination

Transfer quantitatively the test portion into a 1 000 ml one-mark volumetric flask (14.3.3). Add about 900 ml distilled water (analytical grade).

Stir with a magnetic stirrer (1 000 r/min) for 1 h, away from light. Remove the magnetic bar.

Make up to the mark with distilled water. Close with a glass stopper and homogenize.

Take an aliquot part with the 20 ml pipette (14.3.4). Transfer into a 200 ml one-mark volumetric flask. Make up to the mark with distilled water. Close with a glass stopper and homogenize.

Filter the solution, rapidly and away from light, through the membrane (14.3.5) so as to obtain a clear solution.

Adjust the spectrometer (14.3.1) and record the variation in absorbance of the filtered solution between 200 nm and 700 nm using distilled water as the reference liquid. An example of a UV-vis profile is given in Annex C.

14.5 Expression of results

The results are obtained by direct reading of the specific absorbance at three wavelengths, as follows:

$A^{1\%}_{1\,cm}$ (257 nm): absorbance at about 257 nm (λ_{max} of picrocrocin);

$A^{1\%}_{1\,cm}$ (330 nm): absorbance at about 330 nm (λ_{max} of safranal);

$A^{1\%}_{1\,cm}$ (440 nm): absorbance at about 440 nm (λ_{max} of crocins).

1) Millex-LCR is an example of a suitable product available commercially. This information is given for the convenience of users of this document, and does not constitute an endorsement by ISO of this product.

$$A\,{}^{1\,\%}_{1\,\mathrm{cm}}(\lambda_{\max}) = \frac{D \times 10\,000}{m \times (100 - w_{\mathrm{MV}})}$$

where

D is the specific absorbance;

m is the mass, in grams, of the test portion (14.4.1);

w_{MV} is the moisture and volatile matter content, expressed as a percentage mass fraction, of the sample.

14.6 Test report

The test report shall contain at least the following information:

a) the method used, including a reference to this part of ISO 3632 (ISO 3632-2:2010);

b) the results obtained;

c) all operating details not specified or regarded as optional, as well as any incidents which may have influenced the results;

d) the moisture content and volatile matter as determined by the method specified in Clause 7;

e) the particle size of the saffron, if it is in powder form;

f) all information necessary for the complete identification of the sample;

g) the filtration membrane (14.3.5) used.

15 Detection of artificial coloring: identification of synthetic water-soluble acidic colorants — Thin-layer chromatography method

15.1 General

This method is directly applicable: to saffron in powder form, provided that the powder conforms to the requirements of 10.3; and to saffron filaments and cut filaments after crushing and sieving in accordance with 10.2. It allows the detection of the presence of artificial water-soluble acidic colorants.

15.2 Principle

Artificial water-soluble acidic colorants are extracted. Natural pigments of saffron, in particular crocins, are eliminated by successive washes or acidic treatment. Artificial water-soluble acidic colorants are isolated and eluted by chromatography on a polyamide microcolumn, and identified by TLC.

15.3 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

15.3.1 Methanol.

15.3.2 Acetone.

15.3.3 Formic acid, 98 % mass fraction or **glacial acetic acid**.

15.3.4 Ammonia solution, 25 % mass fraction.

15.3.5 Sulfuric acid, 98 % mass fraction.

15.3.6 Sodium hydroxide solution, 40 g/100 ml.

15.3.7 Elution solvent for purification of the methanol and ammonia column.

Add 5 ml ammonia solution (15.3.4) to a 100 ml test tube. Add 95 ml methanol (15.3.1).

15.3.8 Elution solvent mixture.

15.3.8.1 Eluent 1. Dissolve 2 g trisodium citrate in a mixture of 80 ml water and 20 ml ammonia solution (15.3.4).

15.3.8.2 Eluent 2. Dissolve 0,4 g potassium chloride in a mixture of 50 ml *tert*-butanol, 12 ml prioponic acid, and 38 ml water.

15.3.9 Artificial, water-soluble acidic colorants, stock solutions corresponding to 1 g colorant per litre methanol.

The list of colorants indicated as references is not exhaustive.

In each of a series of eight 100 ml beakers (15.4.11), dissolve separately in methanol (15.3.1): 100 mg quinoline yellow (C.I. 47005); sunset yellow S (C.I. 15985); tartrazine (C.I. 19140); amaranth (C.I. 16185), ponceau 4R (C.I. 16255); azorubine (C.I. 14720); orange II (C.I. 15510), and rocelline (C.I. 15620). Transfer each solution separately into a series of eight 100 ml one-mark volumetric flasks (15.4.9). Make up to the mark with methanol and stir. Each solution contains 1 g colorant per litre methanol.

15.3.10 Artificial, water-soluble acidic colorants, working solutions corresponding to 0,1 g colorant per litre methanol.

In a series of eight 100 ml one-mark volumetric flasks (15.4.9), add 10 ml of each stock solution (15.3.9) using a pipette (15.4.8). Make up to the mark with methanol (15.3.1) and stir.

NOTE These solutions are used to individually measure the R_f according to the procedure specified in 15.5.5.

15.3.11 Artificial, water-soluble acidic colorants, reference solutions corresponding to a mixture of colorants with 0,01 g colorant per litre methanol.

In one 100 ml one-mark volumetric flask (15.4.9), add 10 ml of each working solution (15.3.10) using a pipette (15.4.8). Make up to the mark with methanol (15.3.1) and stir.

15.4 Apparatus

Usual laboratory equipment and in particular the following.

15.4.1 SPE purification column for chromatography, solid phase extraction cartridge filled with 125 mg polyamide 6[2)].

15.4.2 Rotary evaporator.

2) The Chromafix® PA No. 731849 Macherey Nagel cartridge is an example of a suitable product available commercially. This information is given for the convenience of users of this document, and does not constitute an endorsement by ISO of this product.

15.4.3 Tabletop centrifuge, rotational frequency up to 4 000 r/min and rotor for 25 ml tubes.

15.4.4 Centrifuge tubes, capacity 25 ml.

15.4.5 Heart-shaped flask, capacity 10 ml.

15.4.6 Vacuum extraction appliance (optional).

15.4.7 Micropipettes, capable of delivering volumes of 100 µl to 1 ml.

15.4.8 One-mark pipette, capacity 10 ml, ISO 648[2] class A.

15.4.9 One-mark volumetric flask, capacity 100 ml, ISO 1042[4] class A.

15.4.10 Test tube, capacity 100 ml.

15.4.11 Beaker, high form, capacity 100 ml.

15.4.12 Syringe, graduated in microlitres, capable of delivering volumes of up to 10 µl.

15.4.13 Pasteur pipette, ISO 7712[5].

15.4.14 Cellulose plate3).

15.4.15 Thin-layer chromatography tank, glass, with a ground glass lid, capable of containing 200 mm × 200 mm plates.

15.4.16 Plastics syringe, made of polyethylene or polypropylene, capacities 10 ml and 2 ml.

15.4.17 Water bath.

15.4.18 pH meter.

15.5 Procedure

15.5.1 Test portion

Weigh, to the nearest milligram, 500 mg saffron in a centrifuge tube (15.4.4) from powder obtained in 10.2 for saffron in filaments and cut filaments and in 10.3 for saffron in powder form.

15.5.2 Extraction of artificial colorants

15.5.2.1 Add 25 ml water, heated to 60 °C, using a graduated pipette (15.4.8). Stir manually for 1 min, ensuring that the saffron powder does not remain in suspension on the sides of the tube. If some remains at the bottom, suspend the saffron once again by stirring with a small spatula. After agitation, allow to rest for 10 min away from light and then stir vigorously again.

15.5.2.2 Balance the tubes and centrifuged them at 4 000 r/min for 10 min (15.4.3).

Transfer the supernatant to a beaker (15.4.11) using a Pasteur pipette (15.4.13). Add 500 µl of formic acid or 2,5 ml glacial acetic acid (15.3.3) to bring the medium to approximately pH 2 (15.4.18).

3) Merck 5730 and Merck 5577 are examples of suitable products available commercially. This information is given for the convenience of users of this document, and does not constitute an endorsement by ISO of this product.

15.5.2.3 If the chromatograms are unacceptably contaminated by peaks due to the natural pigments of saffron, adopt the following alternative procedure to that specified in 15.5.2.2.

Balance the tubes and centrifuge (15.4.3) them at 4 000 r/min for 10 min.

Transfer the supernatant, using a Pasteur pipette (15.4.13), to a 50 ml beaker (15.4.11) and add sulfuric acid (15.3.5) using a micropipette (15.4.7) until the solution attains pH 0,1 (15.4.18).

Heat the solution for 30 min at 100 °C in a water bath (15.4.17). Balance the tubes and centrifuge them at 4 000 r/min for 5 min (15.4.3).

Transfer the supernatant, using a Pasteur pipette (15.4.13), to a 50 ml beaker (15.4.11).

Adjust the solution obtained to pH 2 (15.4.18) with sodium hydroxide solution (15.3.6).

Balance the tubes and centrifuge them at 4 000 r/min for 5 min (15.4.3).

15.5.3 Purification of sample

15.5.3.1 Preparation of purification column and absorption of artificial colorants

Use solid phase extraction cartridge (15.4.1).

Prepare the solid-phase extraction cartridge (15.4.1) with 10 ml water.

Using a 10 ml syringe (15.4.16), pass the saffron extract obtained in 15.5.2.2 or 15.5.2.3 through the cartridge.

15.5.3.2 Elimination of unwanted components

Wash the SPE cartridge successively with 45 ml methanol (15.3.1), 45 ml acetone (15.3.2), and 45 ml methanol (15.3.1), and acidify with 500 µl formic acid (15.3.3) at a constant flow of approximately 6 ml/min to 8 ml/min.

NOTE Acidification of methanol in this washing procedure permits greater recovery.

If the procedure specified in 15.5.2.1 is used, adjust the volume of rinsing agent to 10 ml instead of 45 ml.

15.5.3.3 Elution of artificial colorants

Elute bound colorants with about 10 ml elution solvent (15.3.7) and collect the coloured elute in a heart-shaped flask (15.4.5). Evaporate to dryness on a rotary evaporator (15.4.2) at low pressure and temperature not higher than 40 °C.

Redissolve the residue in 300 µl of methanol dispensed from a micropipette (15.4.7). The length of the washing procedure dramatically decreases if a vacuum extraction appliance (15.4.6) is used. A vacuum extraction appliance facilitates the use of large solvent volumes, but the 6 ml/min to 8 ml/min elution flow rate shall be respected to avoid unacceptable loss in recovery.

15.5.4 Chromatography and detection

Line the walls of the chromatography tank (15.4.15) with filter paper. Pour eluent 1 (15.3.8.1) and eluent 2 (15.3.8.2) into each tank to a depth of about 1 cm and cover with the lid. Leave to settle for 1 h to 2 h in order to saturate the atmosphere inside the tank with solvent vapour.

Add, using the syringe (15.4.12), 10 µl of the methanol residue (15.5.3.3) and 10 µl of the reference solution (15.3.11) 15 mm from the lower edge of the plate (15.4.14) with a distance of 7 mm to 10 mm between each application.

Mark a line parallel to the upper edge of the plate from 70 mm to 150 mm from the application points for eluent 1 and eluent 2.

Put each plate into a chromatography tank. Develop until the solvent front reaches the marked line.

Remove the plates from the tanks and leave to dry under a ventilated hood.

Observe the plates under white light.

NOTE The duration of the chromatography for eluent 1 is about 45 min, for eluent 2 about 8 h.

15.5.5 Calculation

Calculate the R_f values of the reference solution colorants (15.3.11) and sample extract components:

$$R_f = \frac{l_1}{l_2}$$

where

l_1 is the distance travelled by reference or sample spot;

l_2 is the distance travelled by solvent front.

15.5.6 Interpretation of results

Identify any artificial colorant present in the sample extract by comparing the R_f values obtained from the test portion with those of the reference solution.

15.5.7 Expression of results

The test result shall include the limit of detection of the method.

16 Detection of artificial coloring: identification of synthetic water-soluble acidic colorants — High performance liquid chromatography (HPLC)

16.1 General

This test method is suitable for the determination in saffron of the content of the following artificial water-soluble acidic colorants: amaranth, azorubine (carmoisine), orange II, ponceau 4R, quinoline yellow, sunset yellow S, tartrazine, yellow 2G and rocelline.

It is directly applicable to saffron in powder form provided that the powder conforms to the requirements of 10.3 and to saffron filaments and cut filaments after crushing and sieving in accordance with 10.2.

This method is only applicable to the detection and quantification of the selected colorants at the concentration levels listed in Table 3.

16.2 Principle

Artificial water-soluble acidic colorants are extracted. Natural pigments of saffron, in particular crocins, are eliminated by successive washes or acidic treatment. Artificial water-soluble acidic colorants are isolated and eluted by chromatography on a polyamide microcolumn, and identified by HPLC in reverse phase with diode array detector.

16.3 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralised water or water of equivalent purity.

16.3.1 Methanol.

16.3.2 Methanol, HPLC grade.

16.3.3 Acetone.

16.3.4 Acetonitrile, HPLC grade.

16.3.5 Ammonia solution, 25 % mass fraction.

16.3.6 Formic acid, 98 % mass fraction for analysis or **glacial acetic acid**.

16.3.7 Sulfuric acid, 98 % mass fraction.

16.3.8 Sodium hydroxide solution, 40 g/100 ml.

16.3.9 Potassium dihydrogenphosphate, 98 % mass fraction.

16.3.10 Potassium hydroxide, 1 mol/l.

16.3.11 Elution solvent.

In a 100 ml test tube, add 5 ml ammonia solution (16.3.5) and 95 ml methanol (16.3.1).

16.3.12 Artificial, water-soluble acidic colorant solutions.

16.3.12.1 Colorants.

16.3.12.1.1 Quinoline yellow, C.I. 47005.

16.3.12.1.2 Sunset yellow S, C.I. 15985.

16.3.12.1.3 Yellow 2G, C.I. 18965.

16.3.12.1.4 Tartrazine, C.I. 19140.

16.3.12.1.5 Amaranth, C.I. 16185.

16.3.12.1.6 Ponceau 4R, C.I. 16255.

16.3.12.1.7 Azorubine, C.I. 14720.

16.3.12.1.8 Orange II, C.I. 15510.

16.3.12.1.9 Rocelline, C.I. 15620.

16.3.12.2 Standard stock solutions, approximate mass concentration 1 mg/ml.

Weigh, to the nearest 0,1 mg, approximately 100 mg of each colorant (16.3.12.1) separately into a 100 ml one-mark volumetric flask (16.4.12). After adding approximately 80 ml water, mix carefully until complete dissolution. Make up to the mark with water. Calculate the actual concentration in mg per ml solution for each colorant.

16.3.12.3 Diluted standard solutions, approximate mass concentration 10 µg/ml.

Transfer by means of a volumetric glass pipette 1 ml (16.4.9) of each standard stock solution (16.3.12.2) into a series of nine 100 ml one-mark volumetric flasks (16.4.12) and make up to the mark with water. Mix carefully. Calculate the actual concentration, in milligrams per millilitre, of each colorant.

These solutions are used to establish the standard retention time according to the procedure specified in 16.5.3.

16.3.12.4 Dilute multi-standard solutions, approximate mass concentration 20 µg/ml.

Transfer by means of volumetric glass pipettes 1 ml (16.4.9) of each standard stock solution (16.3.12.2) into a 50 ml one-mark volumetric flask (16.4.12) and make up to the mark with water. Mix carefully. Calculate the actual concentration, in milligrams per milliliter, for each colorant.

This solution is used to prepare calibration solutions to cover the concentration range 0 µg/ml to 20 µg/ml. If the expected level exceeds this range, then additional standard solutions shall be prepared.

16.4 Apparatus

16.4.1 Analytical balance, capable of being read to the nearest 0,000 1 g.

16.4.2 Centrifuge tubes, capacity 25 ml.

16.4.3 Tabletop centrifuge, rotational frequency up to 4 000 r/min and rotor for 25 ml tubes.

16.4.4 Solid phase extraction cartridge, filled with 125 mg polyamide 6[2)].

16.4.5 Heart-shaped flask, capacity 10 ml.

16.4.6 Rotary evaporator.

16.4.7 Micropipette, capable of delivering volumes of 100 µl to 1 ml.

16.4.8 Beaker, high form, capacities 25 ml, 100 ml, 500 ml, and 1 l.

16.4.9 Volumetric glass pipette, capacities 1 ml, 10 ml, 25 ml, ISO 648[2] class A.

16.4.10 Pasteur pipette.

16.4.11 Erlenmeyer flask, glass, capacity 1 l.

16.4.12 One-mark volumetric flask, capacities 50 ml, 100 ml, 500 ml, and 1 l, ISO 1042[4] class A.

16.4.13 PTFE membrane filter, pore size 0,45 µm, diameter 13 mm[4)].

16.4.14 pH meter and electrode suitable for 0 to 12 pH measurements.

16.4.15 Test tube capacity 100 ml.

16.4.16 Plastics syringe, made of polyethylene or polypropylene, capacities 10 ml and 2 ml.

16.4.17 Syringe, graduated in microlitres, capable of delivering volumes of up to 50 µl.

16.4.18 Water bath.

16.4.19 Vacuum extraction appliance (optional).

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4) The Pall PTFE Acrodisc membrane is an example of a suitable product available commercially. This information is given for the convenience of users of this document, and does not constitute an endorsement by ISO of this product.

16.4.20 High performance liquid chromatograph, with a diode array detector, capable of measuring absorbance between 300 nm and 700 nm, with pump to allow gradients according to 16.5.4.2.1, thermostatically controlled column compartment (16.5.4.2.1), and recorder whose performances are compatible with all of the apparatus.

16.4.21 Guard column, 10 mm × 4,6 mm with the same phase as the analytical column (16.4.22), but with particle size of 4 µm.

16.4.22 Chromatography column for HPLC, type C_{18}[5].

Stainless steel 150 mm × 4,6 mm, filled with octadecylsilane spherical silica, not end-capped, of particle size 3 µm and porosity 120 nm, surface area 320 m^2/g, and 16 % mass fraction carbon content.

16.4.23 Top vials, capacity 0,6 ml.

16.5 Procedure

16.5.1 Test portion

Weigh, to the nearest milligram, 500 mg saffron in a centrifuge tube (16.4.2) from the powder obtained in 10.2 for saffron in filaments and cut filaments and in 10.3 for saffron in powder form.

16.5.2 Extraction of artificial colorants

16.5.2.1 Add 25 ml water, heated to 60 °C, using a graduated pipette (16.4.9). Stir manually for 1 min, ensuring that the saffron powder does not remain in suspension on the sides of the tube. If some remains at the bottom, suspend the saffron once again using a small spatula. After agitation, allow to rest for 10 min away from light and then stir vigorously again.

16.5.2.2 Balance the tubes and centrifuge them at 4 000 r/min for 10 min (16.4.3).

Transfer the supernatant to a beaker (16.4.8) using a Pasteur pipette (16.4.10). Add 500 µl of formic acid or 2,5 ml glacial acetic acid (16.3.6) to bring the medium to approximately pH 2 (16.4.14).

16.5.2.3 If the chromatograms are unacceptably contaminated by peaks due to the natural pigments of saffron, adopt the following alternative procedure to that specified in 16.5.2.2.

Balance the tubes and centrifuge (16.4.3) them at 4 000 r/min for 10 min.

Transfer the supernatant, using a Pasteur pipette (16.4.10), to a 50 ml beaker (16.4.8) and add sulfuric acid (16.3.7) using a micropipette (16.4.7) until the solution attains pH 0,1 (16.4.14).

Heat the solution for 30 min at 100 °C in a water bath (16.4.18). Balance the tubes and centrifuge them at 4 000 r/min for 5 min (16.4.3).

Transfer the supernatant, using a Pasteur pipette (16.4.10), to a 50 ml beaker (16.4.8).

Adjust the solution obtained to pH 2 with sodium hydroxide solution (16.3.8).

Balance the tubes and centrifuge them at 4 000 r/min for 5 min (16.4.3).

Transfer the supernatant, again using a Pasteur pipette, to a beaker.

The supernatant shall remain free of any particles of saffron so that the solid-phase extraction cartridge used in 16.5.3 does not become clogged. Therefore, repeat the centrifuging step if necessary.

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5) For this part of ISO 3632, the chromatographic conditions and composition of the mobile phase have been established on the basis of a Uptishere Interchrom C18-HDO Interchim column. The use of another type of column may require certain modifications to the mobile phase and chromatographic conditions. This information is given for the convenience of users of this document, and does not constitute an endorsement by ISO of this product.

16.5.3 Purification of sample

16.5.3.1 Preparation of purification column and absorption of artificial colorants

Prepare the solid-phase extraction cartridge (16.4.4) with 10 ml water. Using a 10 ml syringe (16.4.16), pass the saffron extract obtained in 16.5.2.2 or 16.5.2.3 through the cartridge.

16.5.3.2 Elimination of unwanted components

Wash the SPE cartridge successively with 45 ml methanol (16.3.1), 45 ml acetone (16.3.3), and 45 ml methanol (16.3.1), and acidify with 500 µl formic acid (16.3.6) at a constant flow of approximately 6 ml/min to 8 ml/min.

NOTE Acidification of methanol for this washing procedure permits greater recovery.

If the procedure specified in 16.5.2.3 is used, adjust the volume of rinsing agent to 10 ml instead of 45 ml.

16.5.3.3 Elution of artificial colorants

Elute bound colorants with about 10 ml elution solvent (16.3.11), and collect the coloured elute in a heart-shaped flask (16.4.5). Evaporate to dryness on a rotary evaporator (16.4.6) at low pressure and temperature not higher than 40 °C.

Redissolve the residue in 300 µl of water dispensed from a micropipette (16.4.7).

Pass through a PTFE filter (16.4.13) before injecting into an HPLC system.

The length of the washing procedure dramatically decreases if a vacuum extraction appliance (16.4.19) is used. A vacuum extraction appliance facilitates the use of large solvent volumes, but the 6 ml/min to 8 ml/min elution flow rate shall be respected to avoid unacceptable loss in recovery.

16.5.4 Chromatography and detection

16.5.4.1 Eluents.

16.5.4.1.1 Phase A: weigh 1,36 g potassium dihydrogenphosphate (16.3.9), place in a beaker (16.4.8), and add 900 ml water. Adjust to pH 7 (16.4.14) with a solution of potassium hydroxide (16.3.10) and subsequently flush into a 1 l one-mark volumetric flask (16.4.12). Make up to the mark with water.

16.5.4.1.2 Phase B: methanol (16.3.2).

16.5.4.1.3 Phase C: acetonitrile (16.3.4).

16.5.4.2 Procedure

16.5.4.2.1 Chromatographic conditions

Flow: 0,8 ml/min.

Column temperature: 30 °C.

Injection volume: 50 µl.

Chromatographic gradient: see Table 3.

Use the chromatography procedure listed in Table 3.

Inject 50 µl (16.4.17) of the sample extract into the column.

Table 3 — Chromatographic gradients by stage and chromatography procedure

Stage	Time min	Phase A	Phase B	Phase C
Equilibration	10	90	10	0
1	0	90	10	0
2	7	48	52	0
3	10	48	52	0
4	14	0	60	40
5	24	0	60	40
6	25	90	10	0

If problems with chromatographic gradient and flow at high pressure are found, either of the following solutions could be useful.

a) Wash the column as follows:

   1) 50 ml hot water at 40 °C to 60 °C,

   2) 50 ml methanol,

   3) 50 ml acetonitrile,

   4) 50 ml tetrahydrofuran,

   5) 25 ml methanol,

   6) 25 ml mobile phase (initial conditions);

b) Make several injections of a 1 + 1 mixture by volume of dimethylsulfoxide and water.

Plot chromatograms under test conditions at 435 nm (yellow) and 520 nm (red).

16.5.4.2.2 Analysis

Use the diluted multi-standard solutions (16.3.12.4) to perform the system calibration and to quantify the colorants identified. Calibration solutions should cover the concentration range of 0 µg/ml to 20 µg/ml. If the expected levels exceed this range, prepare additional standards.

16.5.5 Interpretation of the results

Identify and confirm artificial colorants in the extract by comparing its retention time and UV-vis spectra between 300 nm to 700 nm with the reference solutions (16.3.12.3). The quantification shall be made using the calibration solutions.

See Annex D for further information.

16.5.6 Expression of results

If a colorant is not detected or confirmed, express the result in the test report as the MRPL (minimum required performance limit) for the artificial water-soluble acidic colorants listed in Table 4.

If colorant is detected and confirmed at a level above the MRPL, express the result in milligrams per kilogram of saffron, to one decimal place.

Table 4 — Minimum required performance limit

Colorant	MRPL mg/kg
Amaranth	1
Azorubine	1
Orange II	2
Ponceau 4R	1
Roccelline	2
Quinoline yellow	1
Sunset yellow	1
Tartrazine	1
Yellow 2G	1

16.6 Test report

The test report shall contain at least the following information:

a) all information needed for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, together with a reference to this part of ISO 3632 (ISO 3632-2:2010);

d) all operating details not specified in this part of ISO 3632 or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained.

Annex A
(informative)

Example for the expression of results for a microscopic examination

For each intersection [number of cellular field/structure], note the number of elements observed. Calculate the percentage of each element, both for the normal and abnormal structures, by dividing the number of observed elements, N_1 to N_n, by the total number of elements, $\sum_{i=1}^{n} N_i$, expressed as a percentage.

An example of a registration table is given in Table A.1.

Table A.1 — Registration table

Element observed	Sum of fields of the five slides: $N_1 \ldots N_n$	Proportion %
Stigmas	N_1	$N_1 \Big/ \sum_{i=1}^{n} N_i$
Styles	N_2	$N_2 \Big/ \sum_{i=1}^{n} N_i$
Pollen grains Stamens Ovary Petals Leaves Stems	N_3	$N_3 \Big/ \sum_{i=1}^{n} N_i$
Hairs Straw Inorganic matter Starch Exogenous plant matter Coloured cells despite illuminating solution Cell anomalies[a]	N_n	$N_n \sum_{i=1}^{n} N_i$
[a] If the cell contents do not diffuse, this can be considered as an anomaly.		

Then use Table A.2 to note the observations of each slide at a magnification of 400 times.

Table A.2 — Count table

Nature of the sample: .....

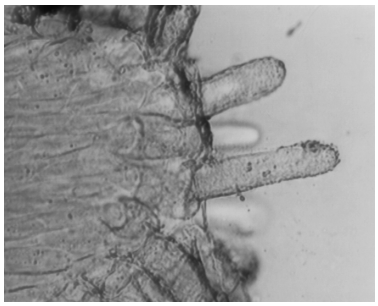
	Field 1	Field 2	Field 3	Field 4	Field 5	Field 6	Field 7	Field 8	Field 9	Field 10
1 Stigmas										
2 Styles										
3 Pollen grains										
4 Stamens										
5 Ovary										
6 Petals										
7 Leaves										
8 Stems										
9 Hairs										
10 Straw										
11 Inorganic matter										
12 Starch										
13 Exogenous plant matter										
14 Coloured cells despite illuminating solution										
15 Cell anomalies[a]										

[a] If the cell contents do not diffuse, this can be considered as an anomaly.

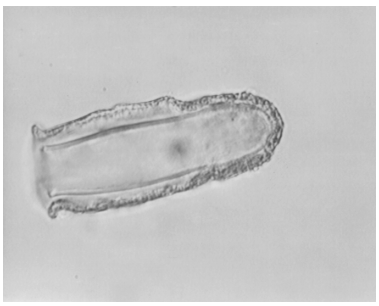
Annex B
(informative)

Photographic references for microscopic identification

See Figures B.1 to B.10

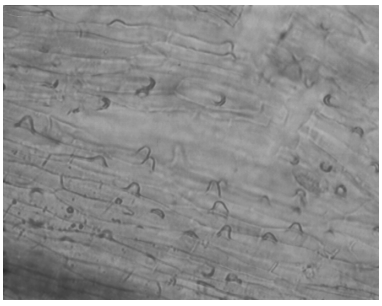


a) Magnification: 100 times

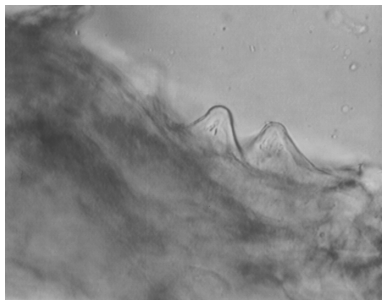


b) Magnification:400 times

Figure B.1 — Stigmatic papillae (top extremity of the stigma)

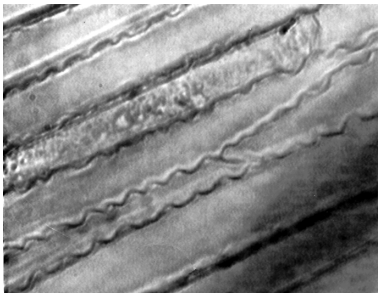


a) Magnification: 100 times

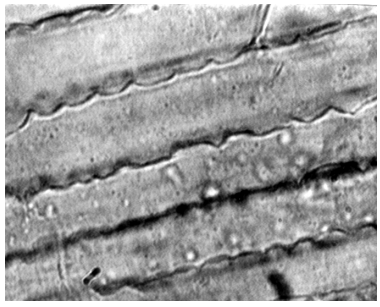


b) Magnification: 400 times

Figure B.2 — Upper epidermis cells with small round papillae

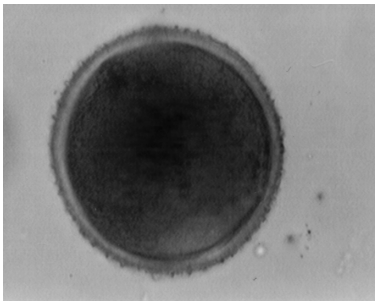


a) Magnification: 100 times

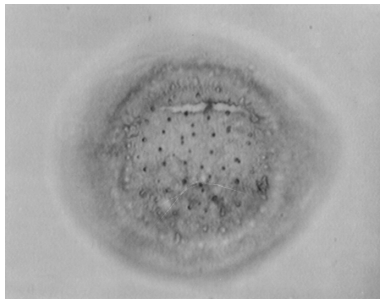


b) Magnification: 400 times

Figure B.3 — Style cells

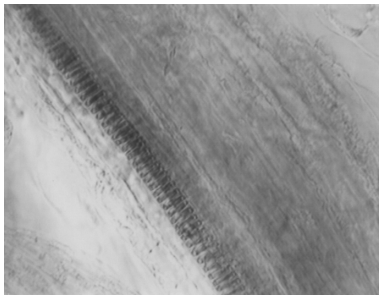


a) Magnification: 100 times

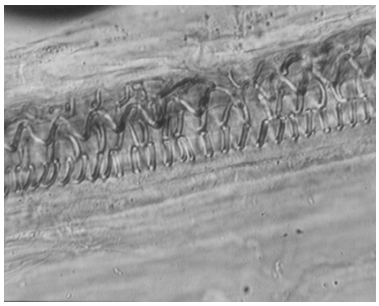


b) Magnification: 400 times

Figure B.4 — Pollen grains

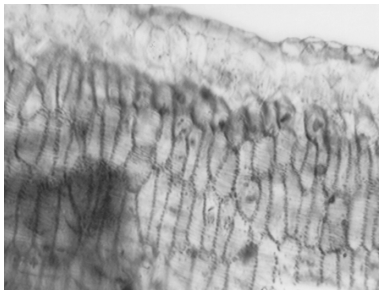


a) Magnification: 100 times

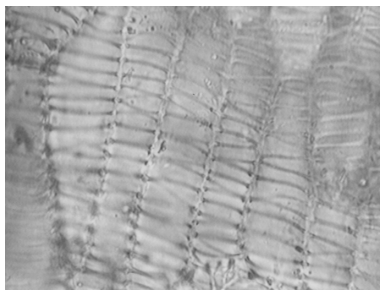


b) Magnification: 400 times

Figure B.5 — Conductor vessels

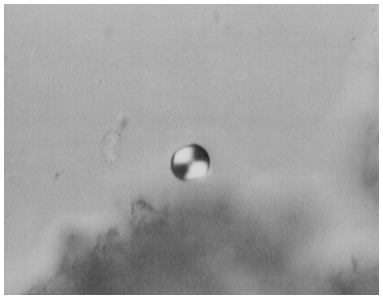


a) Magnification: 100 times

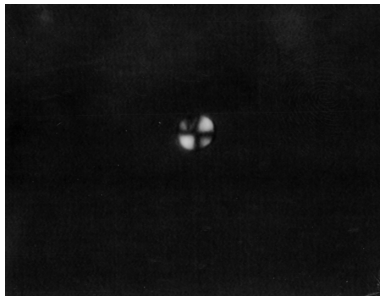


b) Magnification: 400 times

Figure B.6 — Stamens

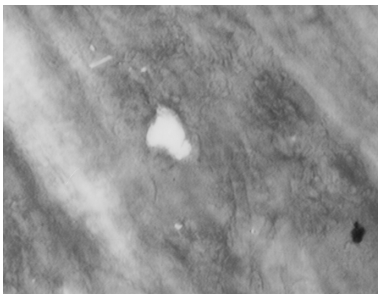


a) Magnification: 100 times

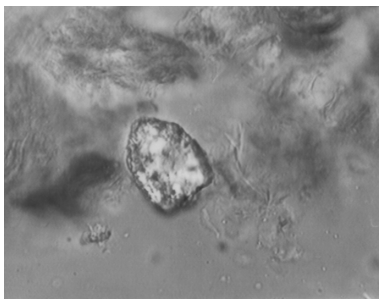


b) Magnification: 400 times

Figure B.7 — Starch grain in polarized light

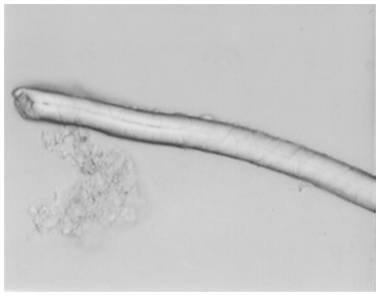


a) Magnification: 100 times

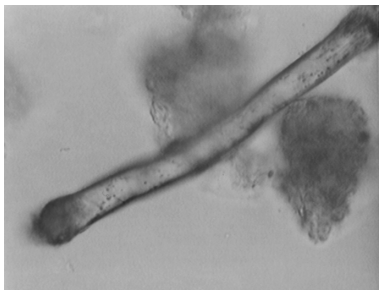


b) Magnification: 400 times

Figure B.8 — Inorganic matter

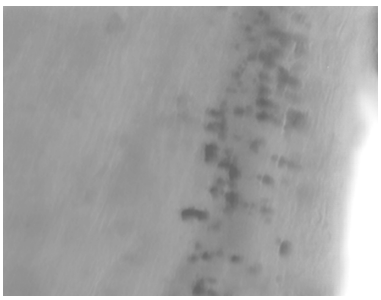


a) Magnification: 100 times

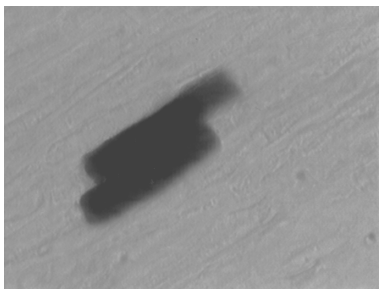


b) Magnification: 400 times

Figure B.9 — Foreign matter



a) Magnification: 100 times



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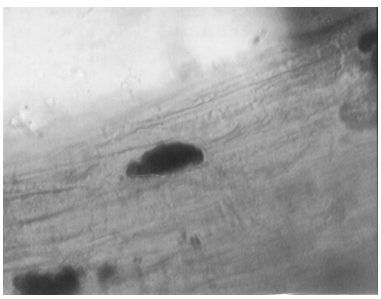

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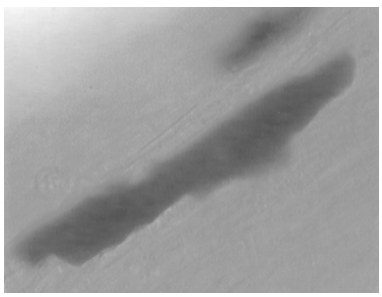


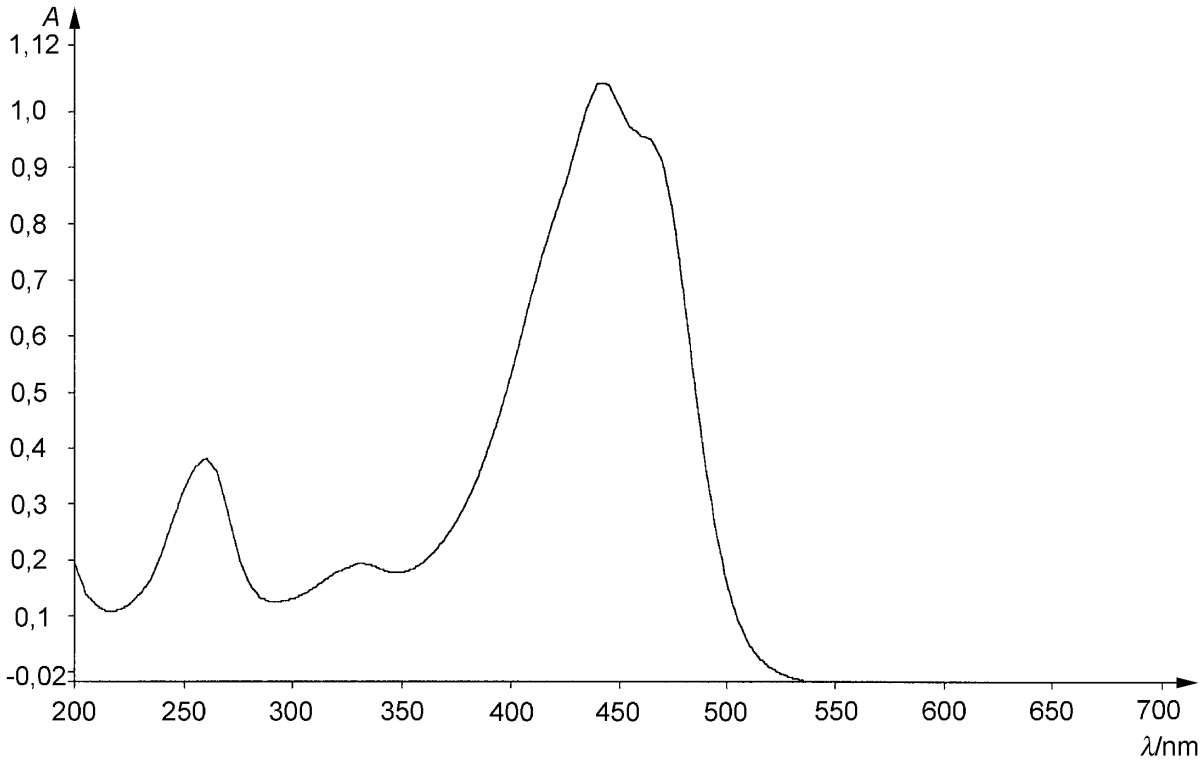
d) Magnification: 400 times

Figure B.10 — Coloured cells despite illuminating solution

Annex C
(informative)

Example of a UV-vis profile of an aqueous extract of saffron

See Figure C.1.



Key

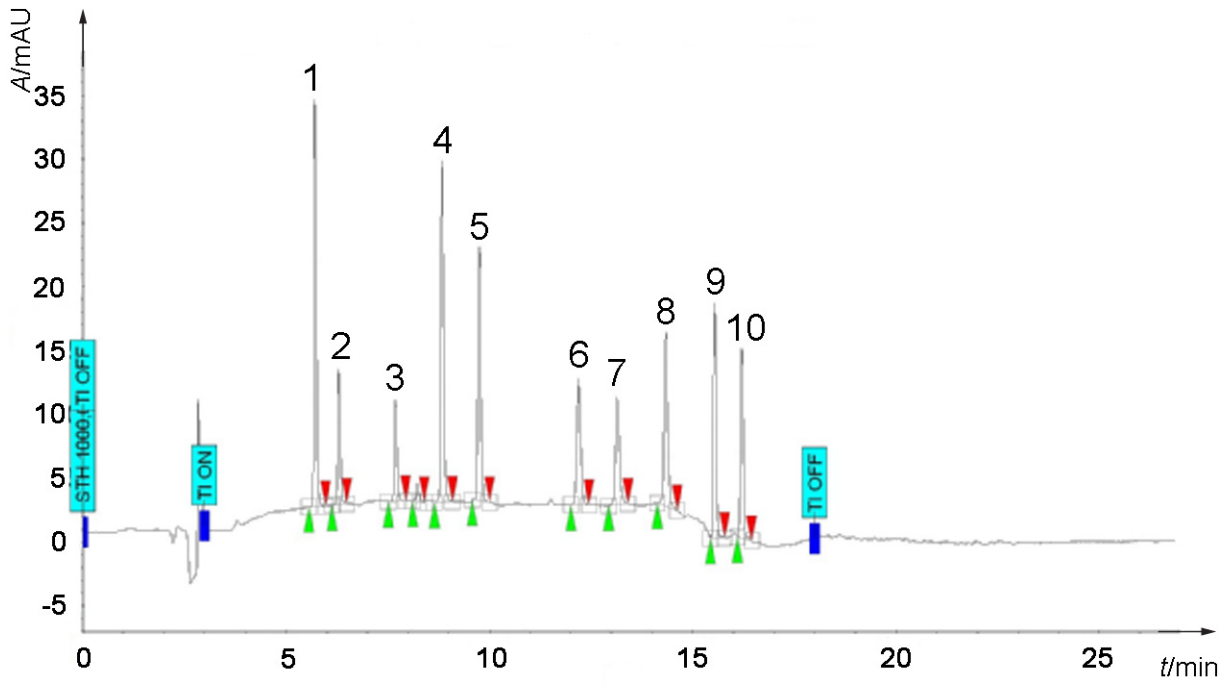
A absorbance

λ wavelength

Figure C.1 — UV-vis spectrum ranging from 200 nm **to** 700 nm **of an aqueous extract of saffron**

Annex D
(informative)

Examples of chromatograms



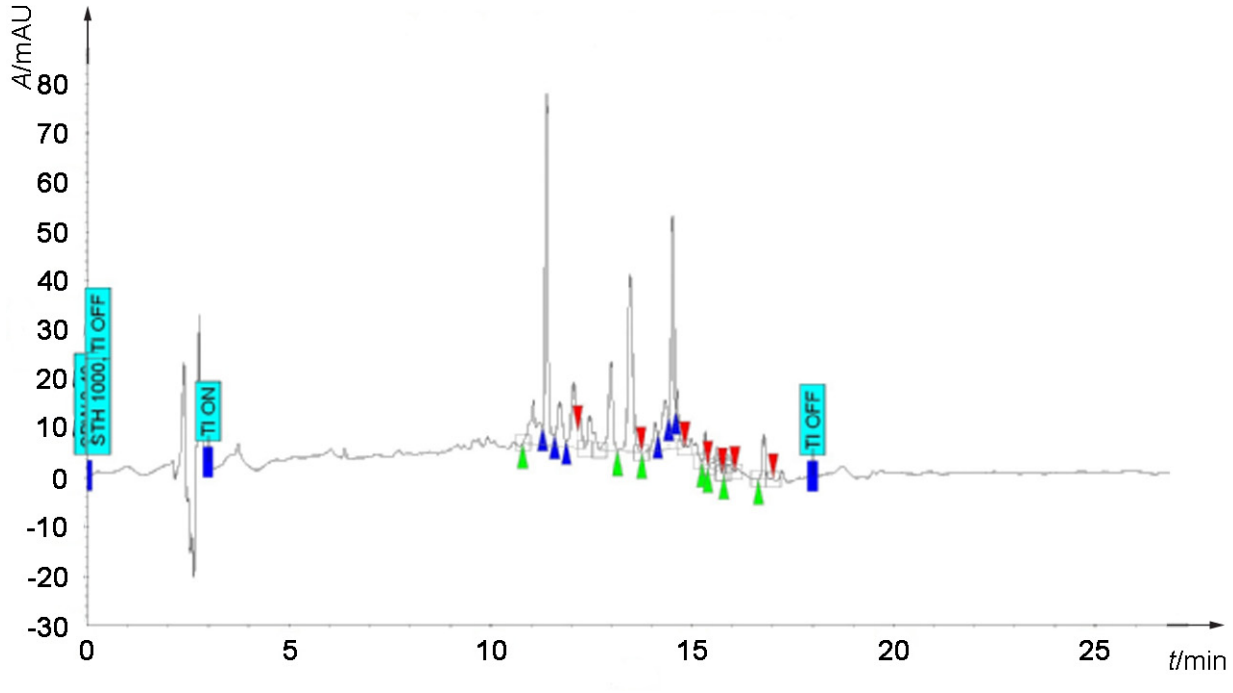
Key

A absorbance

t time

		t
		min
1	tartrazine	5,71
2	amaranth	6,29
3	ponceau 4R	7,69
4	sunset yellow	8,84
5	yellow 2G	9,75
6	azorubin	12,19
7	quinoline yellow (isomer 1)	13,15
8	quinoline yellow (isomer 2)	14,33
9	orange II	15,55
10	rocelline	16,20

Figure D.1 — Chromatogram obtained under test conditions at 440 nm **of** 1 mg/kg **standard**

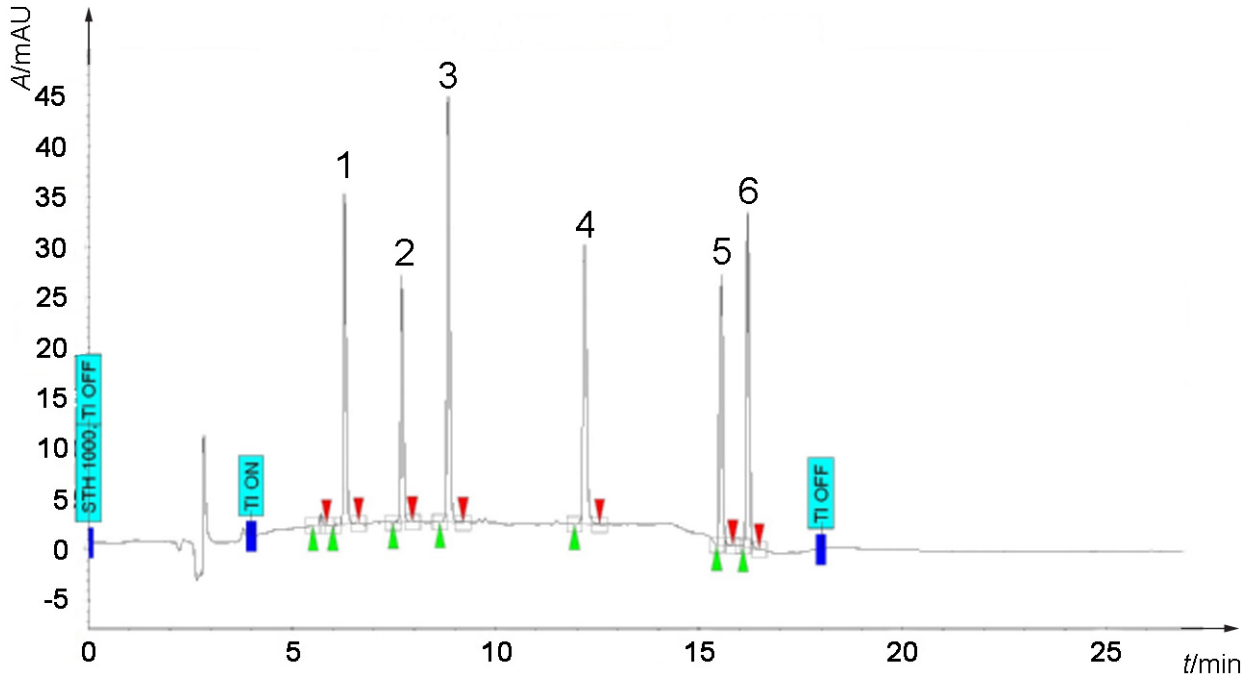


Key

A absorbance

t time

Figure D.2 — Chromatogram obtained under test conditions at 440 nm **of blank sample of saffron**



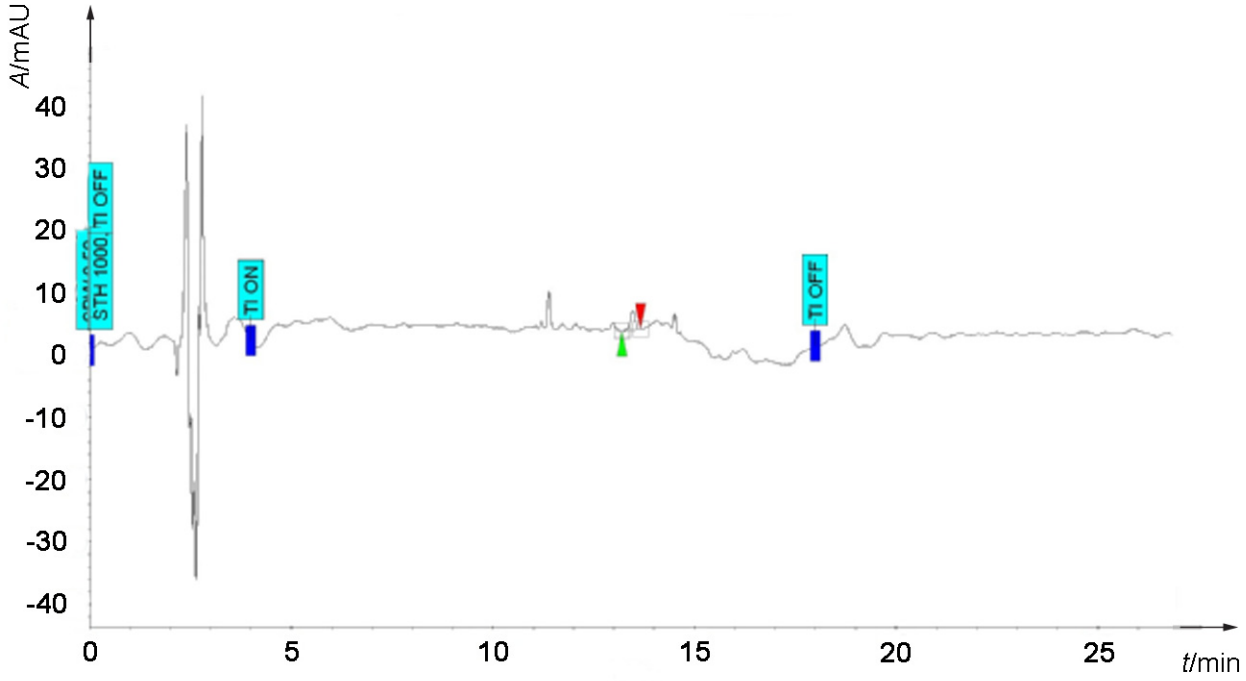
Key

A absorbance

t time

		t
		min
1	amaranth	6,29
2	ponceau 4R	7,69
3	sunset yellow	8,84
4	azorubin	12,19
5	orange II	15,55
6	rocelline	16,20

Figure D.3 — Chromatogram obtained under test conditions at 506 nm **of** 1 mg/kg **standard**

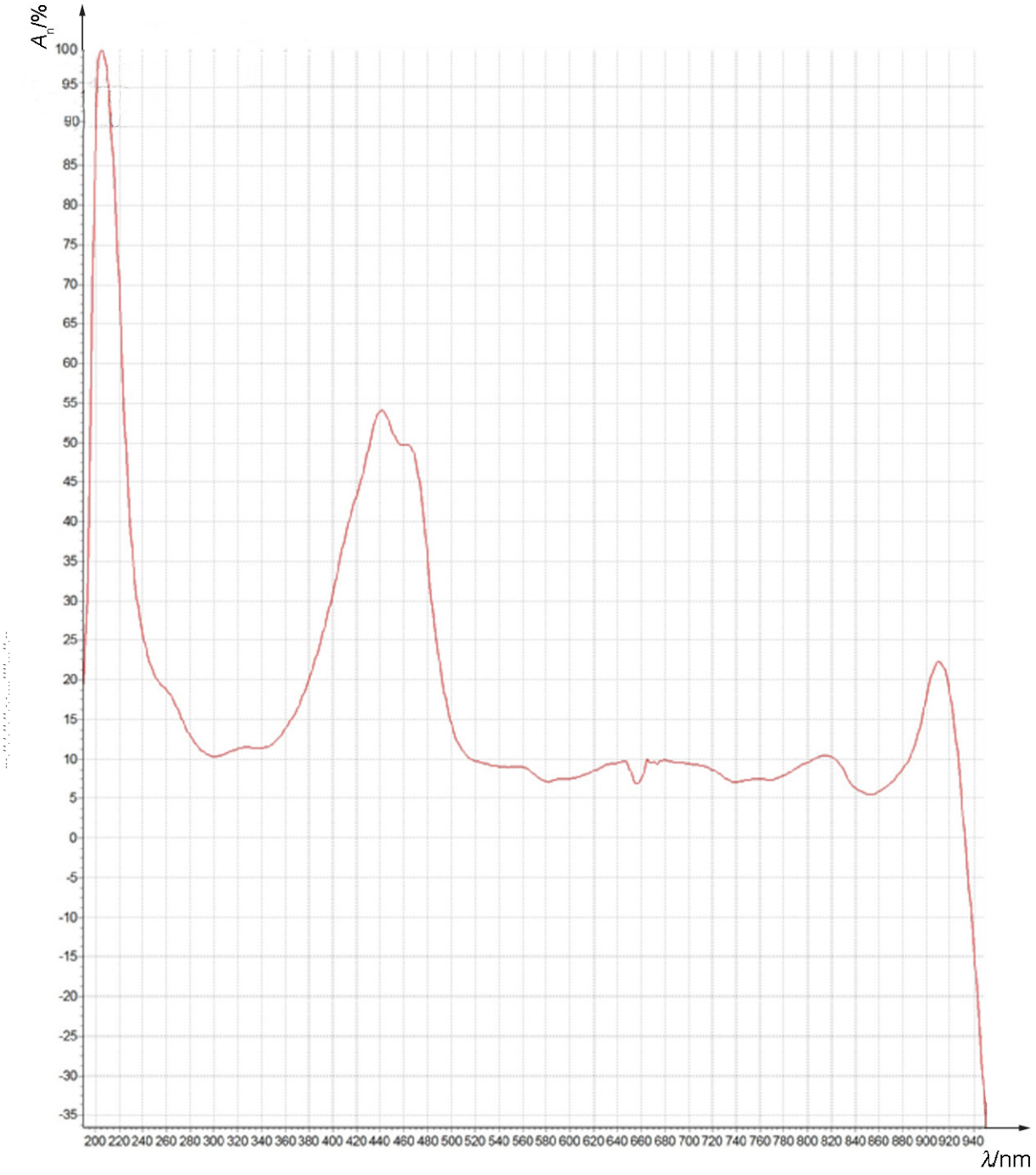


Key

A absorbance

t time

Figure D.4 — Chromatogram obtained under test conditions at 506 nm **of blank sample of saffron**



Key

A_n normalized absorbance

λ wavelength

t_r = 11,700 min

Component name: Matriz

Figure D.5 — Spectrum of peak at 11,70 min **in blank sample of saffron shown in Figure D.2**

Bibliography

[1] ISO 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

[2] ISO 648, *Laboratory glassware — Single-volume pipettes*

[3] ISO 939, *Spices and condiments — Determination of moisture content — Entrainment method*

[4] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

[5] ISO 7712, *Laboratory glassware — Disposable Pasteur pipettes*

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