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Traditional Chinese medicine — *Carthamus tinctorius* flower

*Médecine traditionnelle chinoise — Fleur de carthame des teinturiers (*Carthamus tinctorius*)*

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Foreword

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This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

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

Carthamus tinctorius flower, the dried flower of *Carthamus tinctorius* Linné (Compositae), is a medicinal herb used to treat menstrual problems, cardiovascular disease, pain, and swelling associated with trauma in Asian countries and the Mediterranean region for thousands of years.

There are at least 60 countries worldwide using or producing *Carthamus tinctorius* flower and its products. Major users include India, China, the United States, Iran, Canada, Australia, Republic of Korea, etc. Due to its great demand in the global market, trade in *Carthamus tinctorius* flower has been complicated by adulteration and substitution issues. Dishonest vendors add sand, saline and syrup to increase the weight for sale. Stains such as golden orange II, lemon yellow, and carmine, are sometimes used to make its colour brighter, which can cause health risks. Factors including contamination, packaging and storage conditions also affect the quality of *Carthamus tinctorius* flower.

The establishment of an international standard for *Carthamus tinctorius* flower is therefore necessary to support its quality consistency, clinical effectiveness and safety in international trade.

As national implementation can differ, national standards bodies are invited to modify the values given in 5.4, 5.5, 5.6, 5.7 and 5.8 in their national standards. Examples of national and regional values are given in Annex E.

Traditional Chinese medicine — *Carthamus tinctorius* flower

1 Scope

This document specifies the minimum requirements and test methods for *Carthamus tinctorius* flower that is derived from *Carthamus tinctorius* Linné.

It is applicable to *Carthamus tinctorius* flower that is sold and used as Chinese materia medica.

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 18664, *Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine*

ISO 21371, *Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use*

ISO 22217, *Traditional Chinese medicine —Storage requirements for raw materials and decoction pieces*

ISO 22258, *Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography*

ISO 22283, *Traditional Chinese medicine — Determination of aflatoxins in natural products by LC-FLD*

ISO 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23723, *Traditional Chinese medicine — General requirements for herbal raw material and materia medica*

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
***Carthamus tinctorius* flower**
dried *tubulous flower* (3.2) of *Carthamus tinctorius* Linné (Compositae)

3.2
tubulous flower
flower with a long, thin, straight-sided tube formed of united petals, often separating at the mouth into a flared shape

3.3
tubular corolla
regular and gamopetalous corolla with the petals fused to form a tube

4 Descriptions

Carthamus tinctorius flower is the dried tubulous flower of *Carthamus tinctorius* Linné (Compositae). The flower is collected in summer when it turns from yellow to red, then dried in a shaded area or under the sun, as shown in Figure 1.

Key

A plant with capitulum
B single tubulous flower
C stigma and stamen
1 tubulous flower
2 lobe
3 phyllary
4 tubular corolla
5 stigma
6 stamens

Figure 1 — Structure of *Carthamus tinctorius* flower

5 Quality and safety requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Carthamus tinctorius* flower shall be clean and free from foreign matter including additional pigments;

b) the presence of living insects, mouldy flower and external contaminants which are visible to the naked eye shall not be permitted.

5.2 Morphological features

a) The tubulous flower without the ovary is 1 cm to 2 cm in length.

b) The tubular corolla is orange-red to red, slender, long, and 5-lobed at the apex.

c) The lobe is in the shape of a narrow cord, 5 mm to 8 mm in length.

d) There are 5 stamens and yellowish white anthers are fused into a tube.

e) The stigma is long cylindrical and slightly branches at the apex.

f) The texture is soft.

g) The odour is slightly fragrant; and the taste is slightly bitter.

5.3 Thin-layer chromatography feature

The identification of *Carthamus tinctorius* flower by a thin-layer chromatography shall present spots or bands with the same colour and position corresponding to those of the standard solution.

5.4 Moisture

The mass fraction of moisture should be determined and should not be more than 13,0 %.

5.5 Total ash

The mass fraction of total ash should not be more than 18,0 %.

5.6 Acid-insoluble ash

The mass fraction of acid-insoluble ash should be determined and should not be more than 5,0 %.

5.7 Absorbance

The absorbance of red pigment at 518 nm should be determined and should not be less than 0,20.

5.8 Water-soluble extract

The mass fraction of water-soluble extract should be determined and should not be less than 30,0 %.

5.9 Marker compound(s)

The mass fraction(s) of marker compound(s), such as flavonoids like hydroxysafflor yellow A or kaempferol, should be determined.

5.10 Heavy metals

The mass fractions of heavy metals such as arsenic, mercury, lead and cadmium should be determined.

5.11 Pesticide residues

The mass fractions of pesticide residues should be determined.

5.12 Sulfur dioxide

The mass fraction of sulfur dioxide should be determined.

5.13 Aflatoxins

The mass fraction of aflatoxins should be determined.

6 Sampling

Sampling of *Carthamus tinctorius* flower shall be carried out in accordance with the method specified in ISO 23723.

7 Test methods

7.1 Macroscopic identification

Samples of not less than 200 g are taken from each batch randomly and observed with the naked eye, smelled and tasted.

7.2 Thin-layer chromatography identification

See Annex A for additional information.

7.3 Determination of moisture

The test method specified in ISO 23723 shall apply.

7.4 Determination of total ash

The test method specified in ISO 23723 shall apply.

7.5 Determination of acid-insoluble ash

The test method specified in ISO 23723 shall apply.

7.6 Determination of absorbance of red pigment

See Annex B for additional information.

7.7 Determination of water-soluble extract

The test method specified in ISO 23723 shall apply.

7.8 Determination of marker compound(s)

See Annex C and Annex D for additional information on determination of the mass fractions of hydroxysafflor yellow A and kaempferol.

7.9 Determination of heavy metals

The test method specified in ISO 18664 shall apply.

7.10 Determination of pesticide residues

The test methods specified in ISO 22258 shall apply.

7.11 Determination of sulfur dioxide content

The test method specified in ISO 22590 shall apply.

7.12 Determination of aflatoxins

The test method specified in ISO 22283 shall apply.

8 Test report

For each test method, the test report shall specify the following:

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

b) the sampling method used;

c) the test method(s) used, with reference to this document;

d) the test result(s) obtained;

e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which can have influenced the test result(s);

f) any unusual features (anomalies) observed during the test;

g) the date of the test.

9 Packaging, storage and transportation

The packaging and transportation requirements specified in ISO 23723 shall apply.

The storage requirements specified in ISO 22217 shall apply.

The *Carthamus tinctorius* flower shall be protected from light, moisture, pollution and entry of foreign substances during long-distance delivery.

10 Marking and labelling

The following items shall be marked or labelled on the packages in accordance with the method specified in ISO 21371:

a) product name and plant scientific name;

b) all quality features indicated in Clause 5, determined in accordance with methods specified in Clause 7;

c) gross mass and net mass of the products;

d) country and province/state of origin of the products;

e) date of production, batch number and expiry date of the products;

f) storage and transportation method;

g) any other information requested by the buyer, such as the harvest year and packaging date (if known).

Annex A
(informative)

Thin-layer chromatography (TLC) identification

A.1 Preparation of test solution

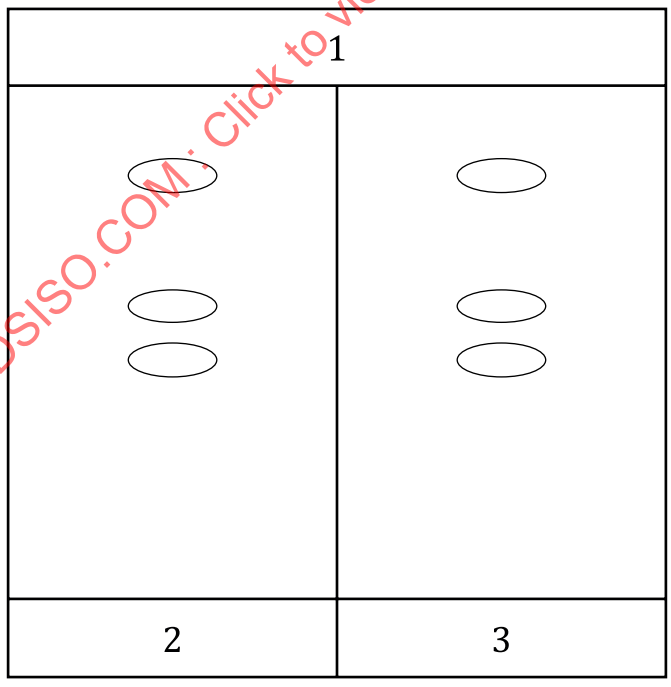
Weigh 0,5 g of powdered sample, add 5 ml of 80 % acetone and shake for 15 min. Filter the mixture and take the filtrate as the test solution.

A.2 Preparation of standard solution

Weigh 0,5 g of powdered reference of medicinal plant material of *Carthamus tinctorius* flower and prepare the standard solution in the same way as A.1.

A.3 TLC identification

Apply 5 µl each of the standard solution and test solution on the same TLC plate (silica gel). Develop with a solution of a mixture of ethyl acetate, formic acid, water and methanol in a volume ratio of 7:2:3:0,4 to a distance of about 10 cm and air-dry the plate. Identify the spots of the test solution by comparing the position and colour with those of the standard solution, which show the same colour and R_f value (the ratio of the solute's distance travelled to the solvent's distance travelled). A typical TLC chromatogram is shown in Figure A.1.



Key

1 top of the plate
2 standard solution
3 test solution

Figure A.1 — TLC chromatograms of *Carthamus tinctorius* flower

Annex B
(informative)

Determination of absorbance of red pigment

The absorbance of red pigment can be determined by the ultraviolet-visible spectrophotometry.

Determination can be conducted using the following steps.

a) Dry the sample in the silica gel drier for 24 h. Weigh 0,25 g of the powdered sample and place it into a conical flask. Add 50 ml of acetone (80 %).

b) Heat the mixture on a water bath at 50 °C for 1,5 h and cool down to room temperature.

c) Filter through a sintered-glass filter (16 µm to 40 µm pore size) and transfer the filtrate to a 100 ml volumetric flask.

d) Wash the residue with acetone (80 %). Combine the solution and make up to the mark with acetone (80 %).

e) Measure the absorbance at 518 nm.

Annex C
(informative)

Determination of hydroxysafflor yellow A content

C.1 Preparation of test solution

Weigh 0,4 g of the powdered sample (passed previously through a sieve of 50 mesh or finer) and place it into a stopper conical flask. Add 50 ml of methanol (25 %). Weigh and sonicate (300 W, 50 kHz) the mixture for 40 min. Cool and weigh again. Replenish the loss of solvent with methanol (25 %) and mix well. Filter the mixture and take the filtrate as the test solution.

C.2 Preparation of reference standard solution

Dissolve the reference standard of hydroxysafflor yellow A with methanol (25 %) to make a solution of 0,13 mg/ml of hydroxysafflor yellow A as the reference standard solution.

C.3 Chromatographic system and high-performance liquid chromatography assay

C.3.1 Column

C.3.1.1 Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent.

C.3.1.2 Size: l = 0,25 m, $\varnothing$ = 4,6 mm.

C.3.2 Mobile phase: mixture of methanol, acetonitrile and 0,7 % phosphoric acid (26:2:72).

C.3.3 Flow rate: 1 ml/min.

C.3.4 Detector: 403 nm.

C.3.5 Injection volume: 10 µl.

C.3.6 System suitability requirements: Perform at least five replicate injections, each using 10 µl of the hydroxysafflor yellow A standard solution. The relative standard deviation (RSD) of the peak area of hydroxysafflor yellow A should not be more than 5,0 %; the RSD of the retention time of hydroxysafflor yellow A peak should not be more than 2,0 %; the column efficiency determined from hydroxysafflor yellow A peak should not be less than 3 000 theoretical plates. The resolution value between hydroxysafflor yellow A peak and the closest peak in the chromatogram of the test solution should not be less than 1,5.

C.4 Content calculation of hydroxysafflor yellow A

C.4.1 The mass fraction (%) of individual component content, w_C, on a sample of dry matter basis, is calculated by Formula (C.1):

$$w_C = \frac{A_{sample} \times m_{std} \times V_{sample} \times p_{std} \times 100}{A_{std} \times m_{sample} \times V_{std} \times p_{sample} \times 1\,000} \quad \text{(C.1)}$$

where

A_{sample} is the peak area of the individual component in the test sample;

A_{std} is the peak area of the individual reference standard;

V_{sample} is the sample extraction volume, in millilitres (50 ml for each sample);

V_{std} is the standard dissolved volume, in millilitres;

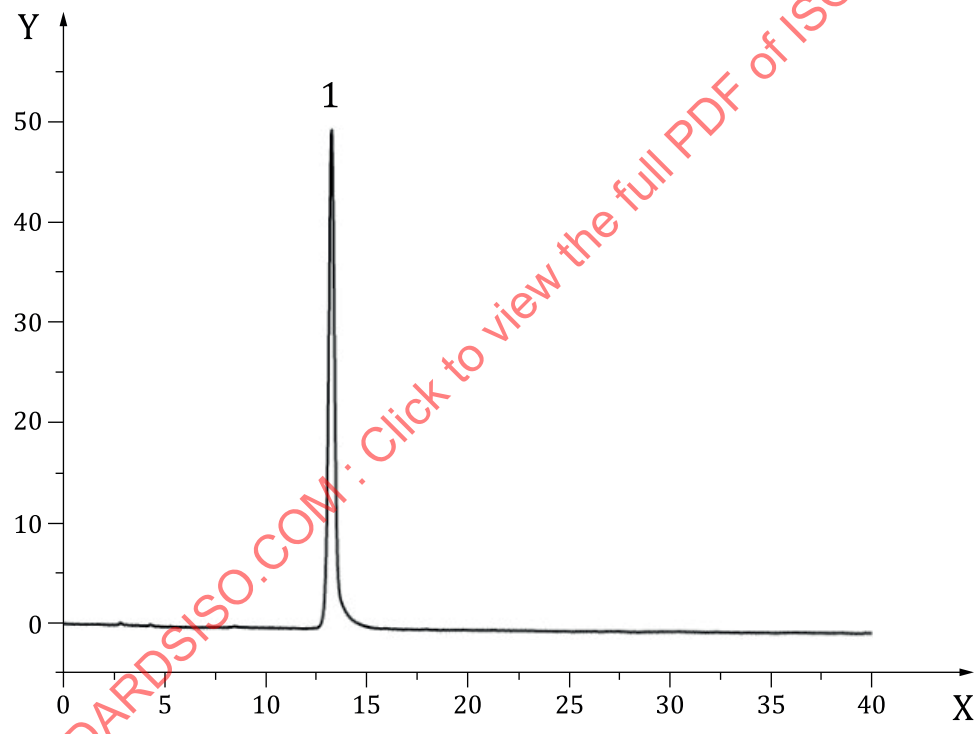
m_{sample} is the mass, in milligrams, of the sample test portion;

m_{std} is the mass, in milligrams, of the individual reference standard;

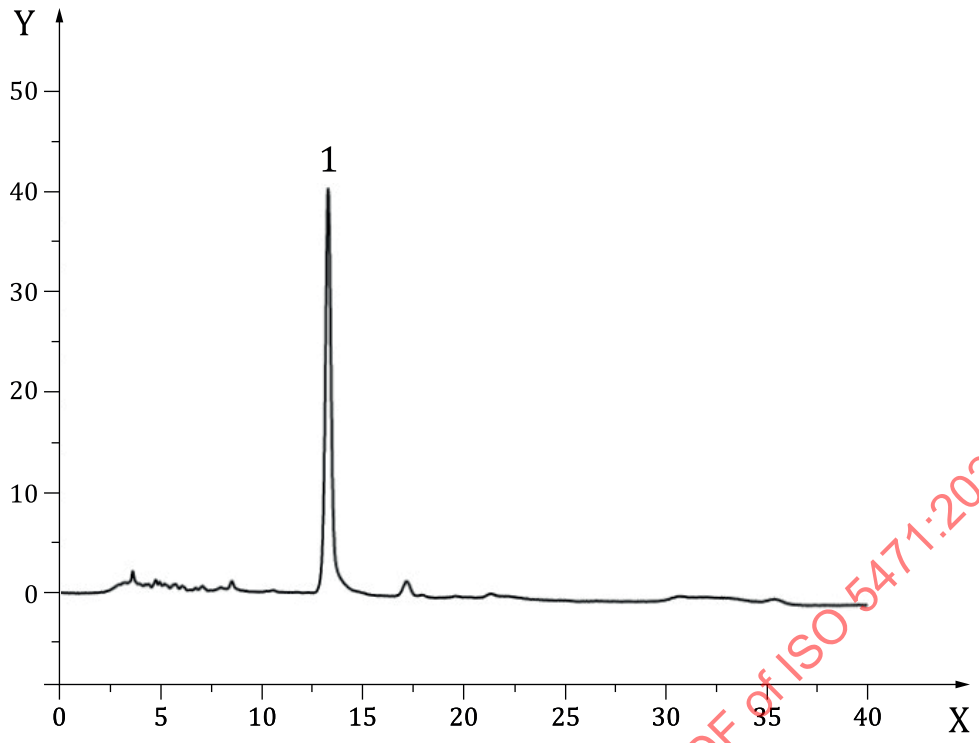
p_{sample} is the mass fraction, in per cent, of the dried test sample;

p_{std} is the mass fraction, in per cent, of the individual reference standard.

C.4.2 HPLC chromatograms of the hydroxysafflor yellow A CRS reference solution and the *Carthamus tinctorius* flower test solution are shown in Figure C.1.



a) Hydroxysafflor yellow A CRS reference solution



b) *Carthamus tinctorius* flower test solution

Key

X mAU

Y min

1 hydroxysafflor yellow A

Figure C.1 — HPLC chromatograms of *Carthamus tinctorius* flower

Annex D
(informative)

Determination of kaempferol content

D.1 Preparation of test solution

Weigh 0,5 g of powdered sample (passed previously through a sieve of 50 mesh or finer) and place it into a stopper conical flask. Add 25 ml of methanol. Weigh and heat the mixture under reflux for 30 min. Cool and weigh again. Replenish the loss of solvent with methanol and mix well. Filter the mixture and transfer accurately 15 ml of the filtrate into a flat bottom flask. Add 5 ml of hydrochloric acid (hydrochloric acid and water, with a volume ratio of 15:22). Mix well and heat the mixture on a water bath for 30 min. Cool down instantly and transfer it into a 25 ml volumetric flask. Make up to the mark with methanol and mix well. Filter the mixture and take the filtrate as the test solution.

D.2 Preparation of reference standard solution

Dissolve reference standard of kaempferol with methanol to make a solution of 9 µg/ml of kaempferol as the reference standard solution.

D.3 Chromatographic system and high-performance liquid chromatography assay

D.3.1 Column

D.3.1.1 Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent.

D.3.1.2 Size: l = 0,25 m, $Ø$ = 4,6 mm.

D.3.2 Mobile phase: mixture of methanol and 0,4 % phosphoric acid (52:48).

D.3.3 Flow rate: 1 ml/min.

D.3.4 Detector: 367 nm.

D.3.5 Injection volume: 10 µl.

D.3.6 System suitability requirements: Perform at least five replicate injections, each using 10 µl of the kaempferol standard solution. The relative standard deviation (RSD) of the peak area of kaempferol should not be more than 5,0 %; the RSD of the retention time of kaempferol peak should not be more than 2,0 %; the column efficiency determined from kaempferol peak should not be less than 3 000 theoretical plates. The resolution value between kaempferol peak and the closest peak in the chromatogram of the test solution should not be less than 1,5.

D.4 Content calculation of kaempferol

D.4.1 The mass fraction of individual component content, w_C, on a sample of dry matter basis, is calculated by Formula (D.1):

$$w_C = \frac{A_{sample} \times m_{std} \times V_{sample} \times p_{std} \times 100}{A_{std} \times m_{sample} \times V_{std} \times p_{sample} \times 1\,000} \quad \text{(D.1)}$$

where

A_{sample} is the peak area of the individual component in the test sample;

A_{std} is the peak area of the individual reference standard;

V_{sample} is the sample extraction volume, in millilitres (50 ml for each sample);

V_{std} is the standard dissolved volume, in millilitres;

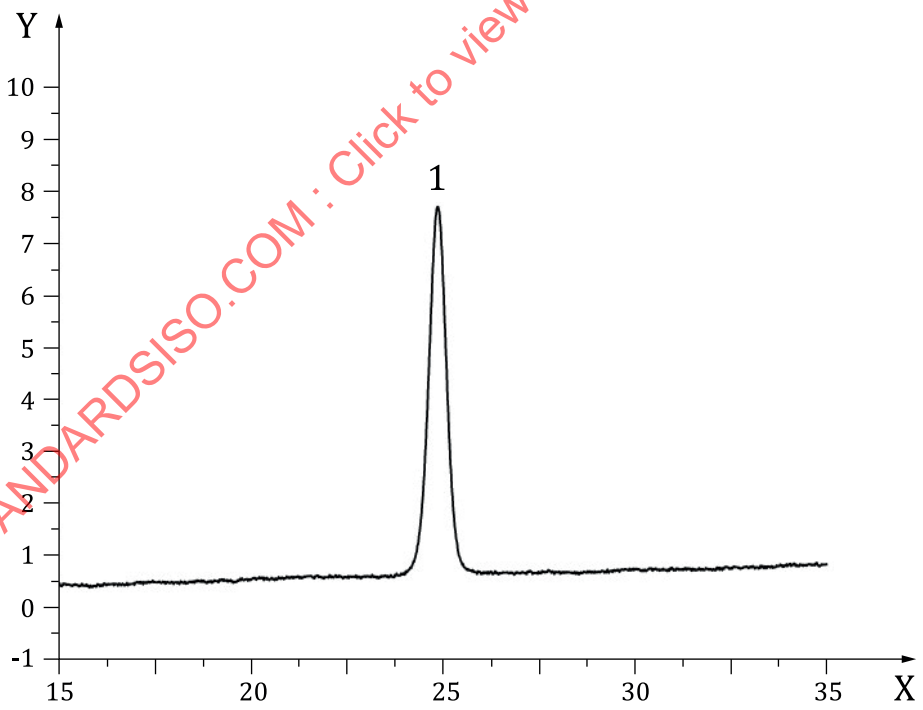
m_{sample} is the mass, in milligrams, of the sample test portion;

m_{std} is the mass, in milligrams, of the individual reference standard;

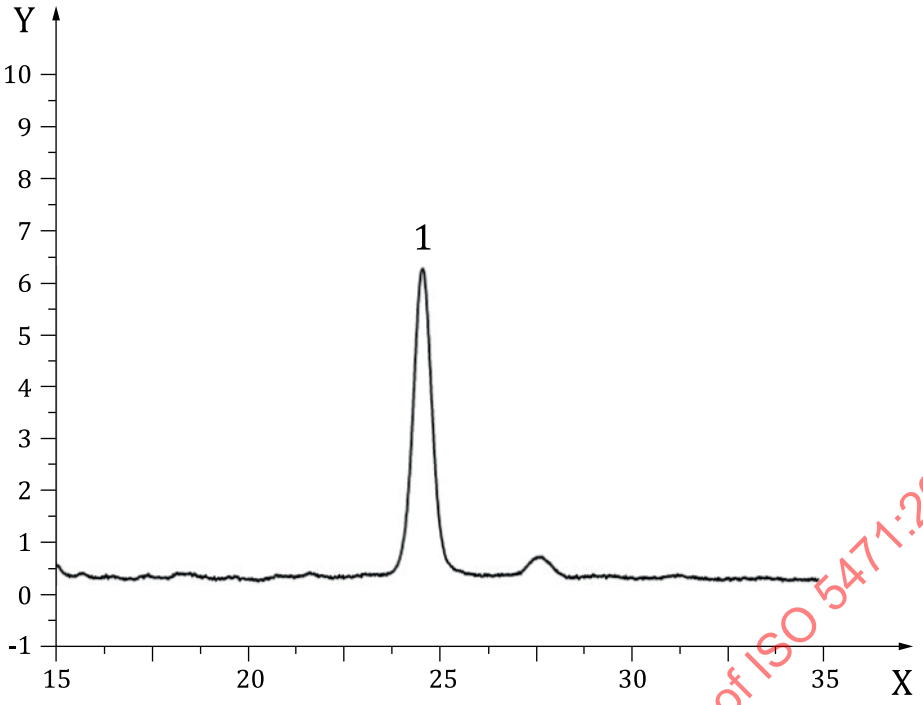
p_{sample} is the mass fraction in per cent of the dried test sample;

p_{std} is the mass fraction in per cent of the individual reference standard.

D.4.2 HPLC chromatograms of the kaempferol CRS reference solution and the *Carthamus tinctorius* flower test solution are shown in Figure D.1.



a) Kaempferol CRS reference solution



b) *Carthamus tinctorius* flower test solution

Key

X min
Y mAU
1 kaempferol

Figure D.1 — HPLC chromatograms of *Carthamus tinctorius* flower