Croci Stigma

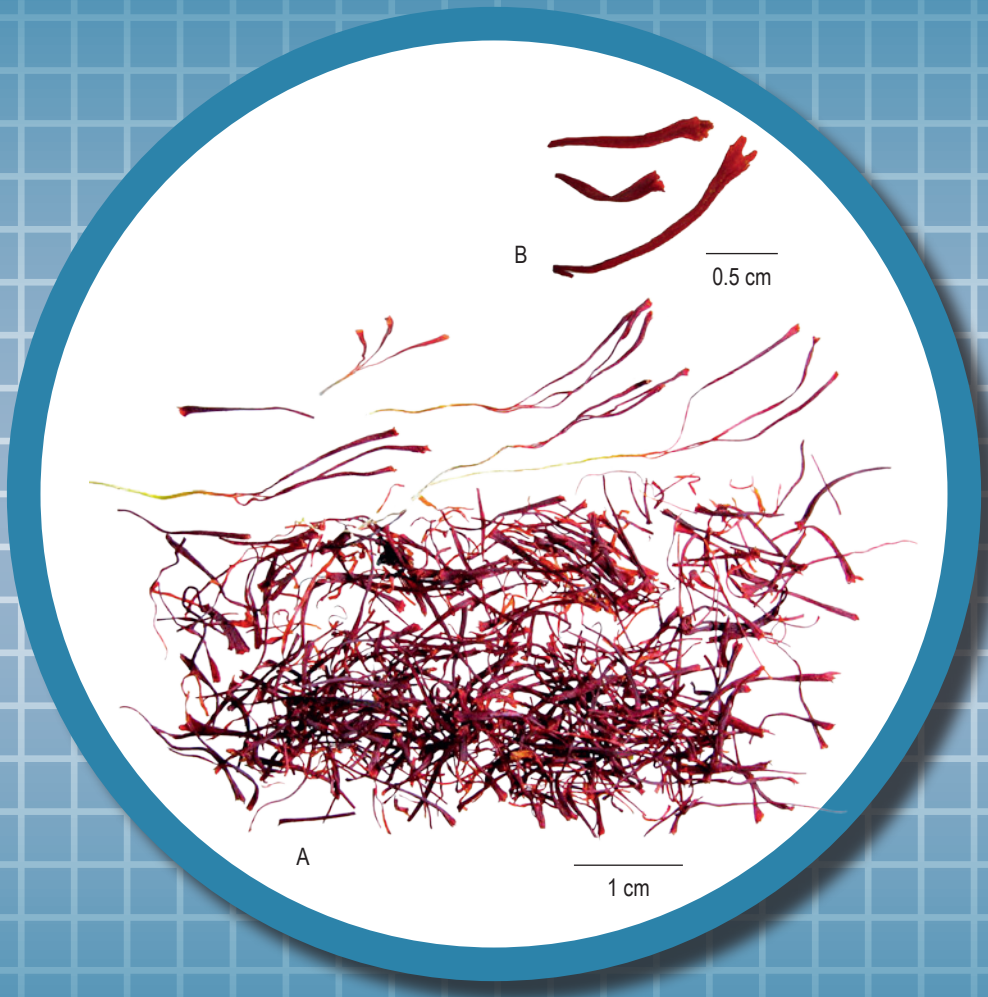


Figure 1 A photograph of Croci Stigma

A. Croci Stigma B. Magnified branches of stigma

1. NAMES

Official Name: Croci Stigma

Chinese Name: 西紅花

Chinese Phonetic Name: Xihonghua

2. SOURCE

Croci Stigma is the dried stigma of *Crocus sativus* L. (Iridaceae). The flower is collected in the morning at flowering stage, the stigma removed and gathered, then dried under the sun or in shaded area; or dried at ambient temperature to obtain Croci Stigma.

3. DESCRIPTION

Linear, 3-branched, 2-3.5 cm long, dark red or reddish-brown. The upper part relatively broader and slightly flattened, the margins of apex irregularly dentate, with a short slit at the inner side, sometimes a small piece of yellow style remained at the lower end. Texture lax, soft and light in weight, without oily lustre; fragile and easily broken after dried. Odour characteristic and slightly irritative; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour orangish-red. Epidermal cells long stripe-shaped in surface view, subsquare, subrounded or subrectangular in section view, wall thin, slightly sinuous, sometimes outer wall protruding or showing papillae. Terminal epidermal cells of stigma tubular protrusions, 18-56 μm in diameter. Fragments of style consist of rectangular parenchymatous cells, arranged tightly, lumen containing many fine crystals of calcium oxalate. Vessels mostly annular, relative small, presented in fragments of style or stigma, spiral vessels sometimes visible. Pollen grains subspheroidal, 35-145 μm in diameter, outer wall almost smooth or with fine spiny exine, containing many minute granular inclusions (Fig. 2).

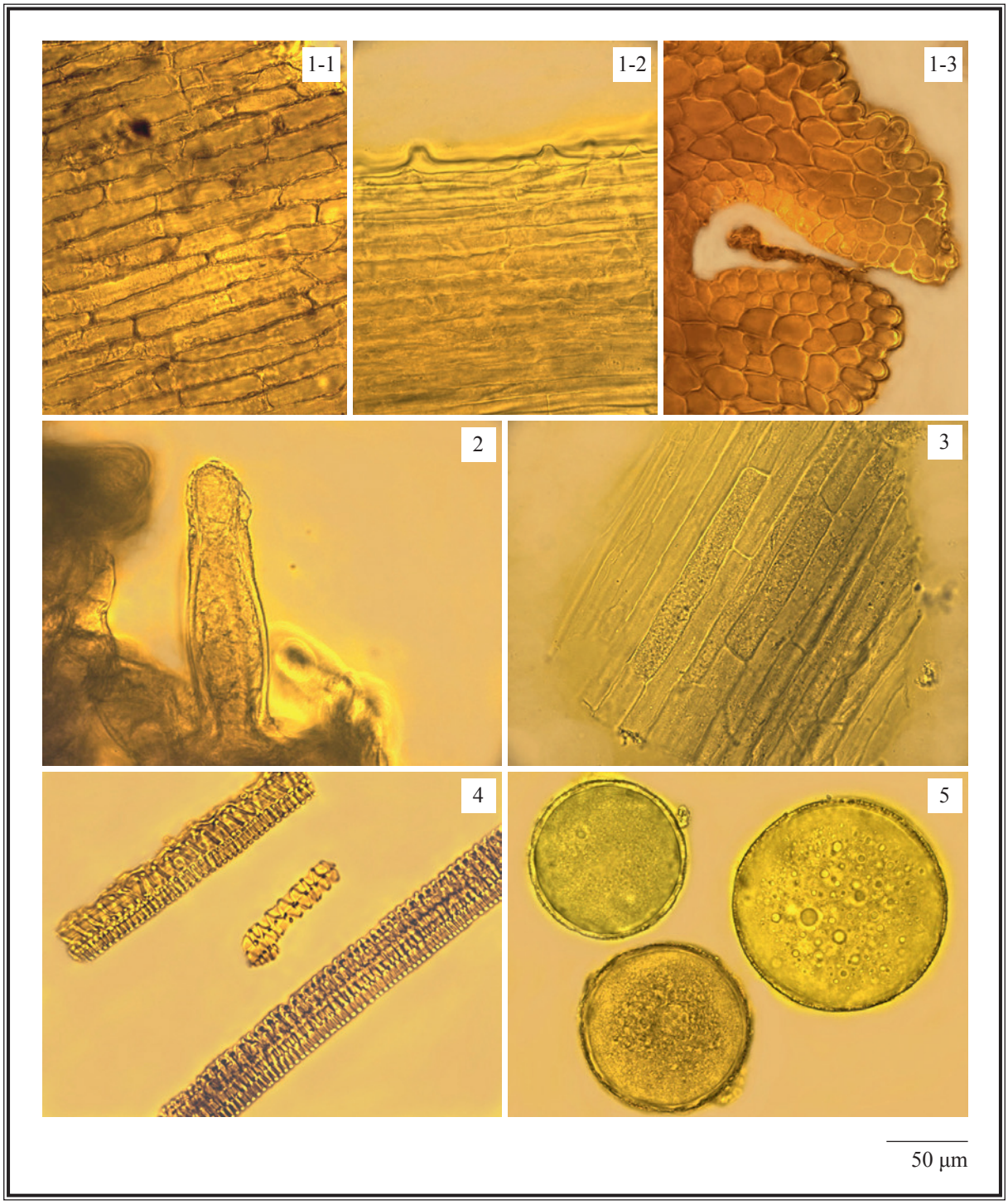


Figure 2 Microscopic features of powder of Croci Stigma (under the light microscope)

1. Epidermal cells (1-1 in surface view, 1-2 in lateral view, 1-3 in section view)
2. Tubular epidermal cell 3. Parenchymatous cells of style 4. Vessels 5. Pollen grains

4.2 Thin-Layer Chromatographic Identification *[Appendix IV(A)]*

Standard solutions

Crocin I standard solution

Weigh 0.5 mg of crocin I CRS (Fig. 3) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with methanol (50%).

Crocin II standard solution

Weigh 0.5 mg of crocin II CRS (Fig. 3) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with methanol (50%).

Developing solvent system

Prepare a mixture of ethyl acetate, isopropanol, water and formic acid (65:35:20:1, v/v).

Test solution

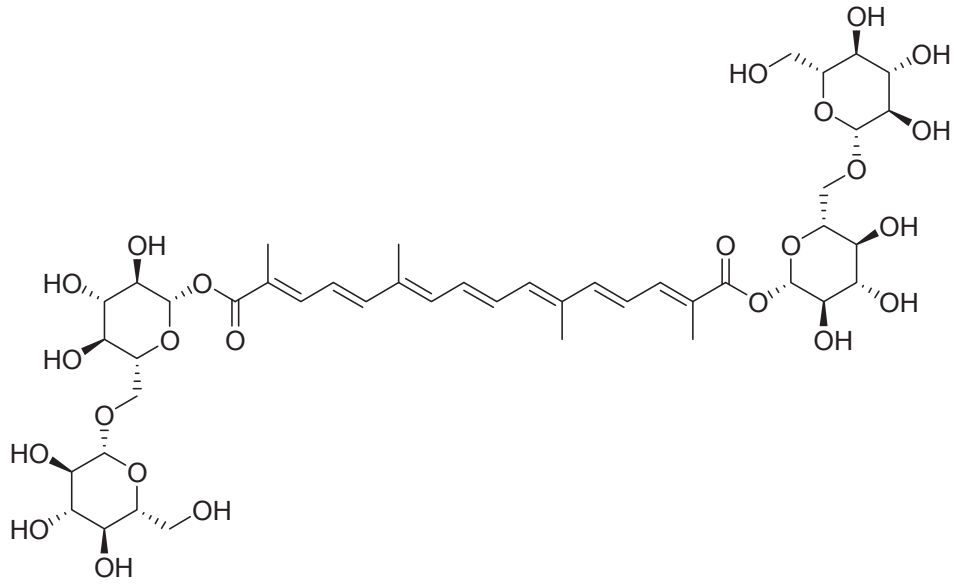
Weigh 0.1 g of the powdered sample and place it in a 25-mL conical flask wrapped in aluminium foil, then add 10 mL of methanol. Sonicate (100 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately crocin I standard solution, crocin II standard solution and the test solution (10 µL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 7 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of crocin I and crocin II.

(i)



(ii)

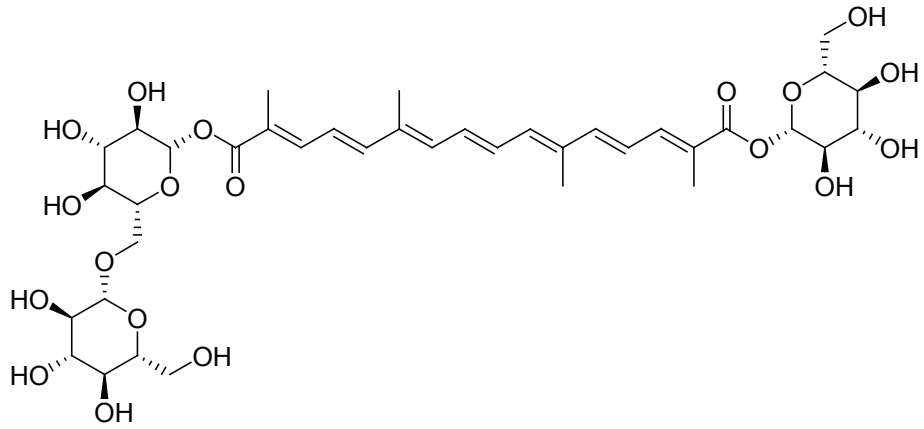


Figure 3 Chemical structures of (i) crocin I and (ii) crocin II

4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Crocin I standard solution for fingerprinting, Std-FP (70 mg/L)

Weigh 1.4 mg of crocin I CRS and place it in a 20-mL amber glass volumetric flask. Make up to the mark with methanol (50%).

Crocin II standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of crocin II CRS and place it in a 50-mL amber glass volumetric flask. Make up to the mark with methanol (50%).

Test solution

Weigh 0.04 g of the powdered sample and place it in a 50-mL centrifuge tube wrapped in aluminium foil, then add 40 mL of methanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL amber glass volumetric flask. Make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 30	90 → 50	10 → 50	linear gradient
30 – 60	50 → 40	50 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of crocin I Std-FP and crocin II Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of crocin I and crocin II should not be more than 5.0%; the RSD of the retention times of crocin I and crocin II peaks should not be more than 2.0%; the column efficiencies determined from crocin I and crocin II peaks should not be less than 80000 theoretical plates.

The *R* value between peak 3 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 4).

Procedure

Separately inject crocin I Std-FP, crocin II Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of crocin I and crocin II peaks in the chromatograms of crocin I Std-FP, crocin II Std-FP and the retention times of the four characteristic peaks (Fig. 4) in the chromatogram of the test solution. Identify crocin I and crocin II peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of crocin I Std-FP and crocin II Std-FP. The retention times of crocin I and crocin II peaks in the chromatograms of the test solution and the corresponding Std-FP should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the four characteristic peaks of Croci Stigma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Croci Stigma extract

Peak No.	RRT	Acceptable Range
1 (picrocrocin)	0.53	± 0.05
2	0.65	± 0.05
3 (crocin I)	0.86	± 0.03
4 (marker, crocin II)	1.00	-

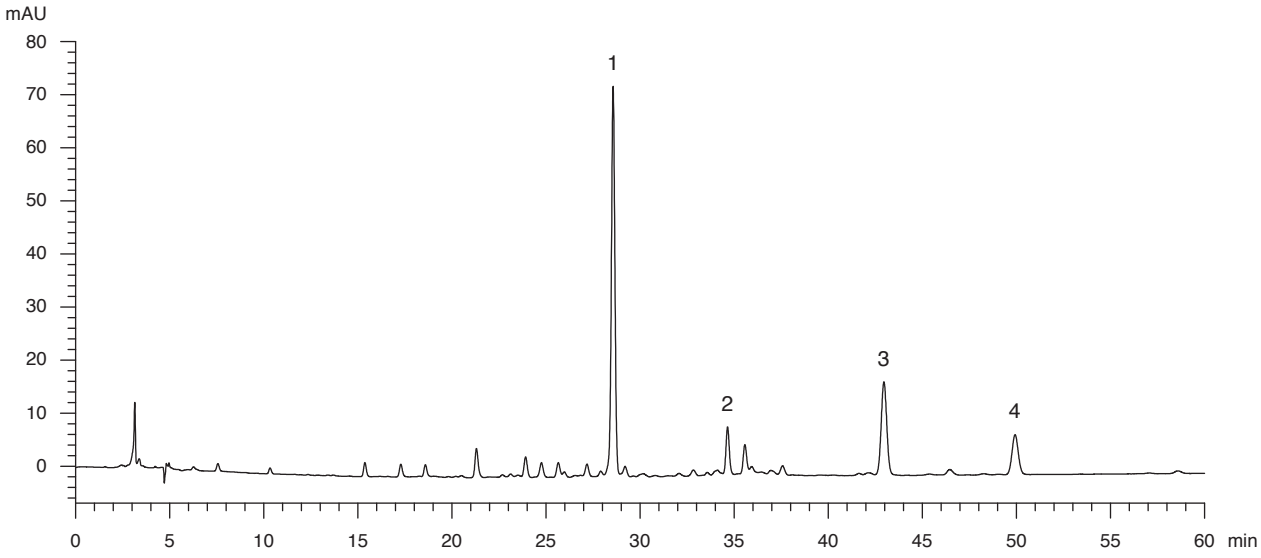


Figure 4 A reference fingerprint chromatogram of Croci Stigma extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 4).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

5.8 Absorbance *(Appendix XV)*

Procedure

Weigh accurately 0.03 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 70 mL of methanol to a 250-mL round-bottomed flask. Perform the soxhlet extraction for about 5 h. Cool down to room temperature. Transfer the extract to a 100-mL volumetric flask and make up to the mark with methanol. Pipette 5 mL of the solution into a 50-mL volumetric flask and make up to the mark with methanol. Measure the absorbance of the solution at 432 nm. The absorbance should not be less than 0.50.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 46.0%.
Ethanol-soluble extractives (hot extraction method): not less than 50.0%.

7. ASSAY

Carry out the method as directed in Appendix IV(B).

Standard solution

Mixed crocin I and crocin II standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of crocin I CRS and 2.0 mg of crocin II CRS, and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol (50%).

Mixed crocin I and crocin II standard solution for assay, Std-AS

Measure accurately the volume of the mixed crocin I and crocin II Std-Stock, dilute with methanol (50%) to produce a series of solutions of 0.5, 2, 10, 20, 40 mg/L for both crocin I and crocin II.

Test solution

Weigh accurately 0.01 g of the powdered sample and place it in a 50-mL centrifuge tube wrapped in aluminium foil, then add 40 mL of methanol (50%). Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL amber glass volumetric flask. Make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (440 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (50:50, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed crocin I and crocin II Std-AS (10 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of crocin I and crocin II should not be more than 5.0%; the RSD of the retention times of crocin I and crocin II peaks should not be more than 2.0%; the column efficiencies determined from crocin I and crocin II peaks should not be less than 3000 and 4000 theoretical plates respectively.

The *R* value between crocin I peak and the closest peak; and the *R* value between crocin II peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed crocin I and crocin II Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of crocin I and crocin II against the corresponding concentrations of the mixed crocin I and crocin II Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify crocin I and crocin II peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed crocin I and crocin II Std-AS. The retention times of crocin I and crocin II peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of crocin I and crocin II in the test solution, and calculate the percentage contents of crocin I and crocin II in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 10% of the total content of crocin I ($C_{44}H_{64}O_{24}$) and crocin II ($C_{38}H_{54}O_{19}$), calculated with reference to the dried substance.