

See description under TESTS

Colour reactions

Passes test
See description under TESTS

PURITY

Synthetic dyes

Passes test
See description under TESTS

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

IDENTIFICATION TESTS

Thin layer chromatography
(Vol. 4)

Activate some silica gel for 1 h at 110° and prepare a TLC plate. Prepare an 0.02% solution of the sample in dimethylformamide and apply 20 µl to the plate. Allow to dry and develop using a mixture of n-butanol, acetic acid and water (4:1:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Carthamin appears as a red spot with an R_f value of about 0.40.

Colour reactions

Dissolve 10 mg of the sample in 50 ml water. The colour of the solution is red. Add alkali to raise the pH to above 7. The colour changes to orange-yellow.

To 0.05 g of the sample add 2 ml of 5% phosphoric acid and heat for 1 h on a water bath. After cooling, filter and wash the residue with 3 ml of water. Combine the filtrate and the washings. Neutralize the combined solution with sodium hydroxide TS, add 5 ml of Fehling's TS and heat on a water bath for 10 min. A red precipitate is produced.

PURITY TESTS

Synthetic dyes

Basic dyes: To 1 g of the sample add 100 ml of 1% sodium hydroxide solution, and mix well. Extract 30 ml of this solution with 15 ml of ether. Then extract the ether layer twice with dilute acetic acid (5 ml); the dilute acetic acid layer does not contain any colour.

Acidic dyes: To 1 g of the sample add 1 ml of ammonia TS and 8 ml of water, and shake well. Discard an oily layer when separated. Proceed as directed in *Paper Chromatography (Ascending Chromatography)* using 20 µl of the solution as the sample solution, and a mixture of pyridine and ammonia TS (2:1 by volume) as the developing solvent. Stop the development when the solvent front has advanced about 15 cm from the point of application. No spot is observed at the solvent front after drying under daylight. If any spot is observed, it should be decolourized when sprayed with a solution of stannous chloride in hydrochloric acid (2 in 5).

METHOD OF ASSAY Transfer about 0.01 g of the sample, accurately weighed, in a 300-ml ground stoppered flask, add 150 ml of dimethylformamide (DMFA), dissolve by shaking occasionally and allow stand for 2 hours. Filter this solution through a glass filter into a 200-ml volumetric flask. Wash the flask and filter with two 25-ml portions of DMFA, combine the filtrate and the washings, add DMFA to volume and mix. Dilute if necessary. Determine the absorbance (A) at the maximum absorbance in the range of 525-535 nm using a 1-cm cell with DMFA as a blank and calculate the percent of colouring matter (P) with the following formula/taking any additional dilution into account:

$$P = \frac{A}{992} \times \frac{200}{W}$$

where

W = weight of sample in g

$A_{1\text{ cm}}^{1\%}$ of carthamin = 992

λ_{max} = 525 - 535 nm