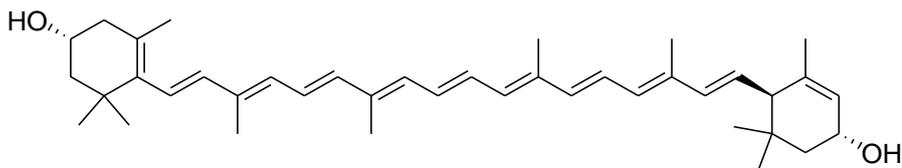


## Lutein



$C_{40}H_{56}O_2$

Mol. Wt. 568.9

Lutein is  $\beta$ - $\epsilon$ -Carotene-3,3'-diol (3R,3'R,6'R). It is the purified fraction obtained from saponification of the oleoresin of *Tagetes erecta* L.

Lutein contains not less than 80.0 per cent of total carotenoids calculated as  $C_{40}H_{56}O_2$ . It contains not less than 74.0 per cent of lutein and not more than 8.5 per cent of zeaxanthin, both calculated as  $C_{40}H_{56}O_2$  on the anhydrous basis.

**Category.** Antioxidant

**Description.** A red crystalline powder.

### Identification

A. When examined in the range 300 nm to 700 nm (2.4.7), the test solution prepared in the test of content of total carotenoids shows the ratio of the absorbance at 446 nm to that at 474 nm is between 1.09 to 1.14.

B. In the test of Content of lutein, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

#### Content of Total Carotenoids

*NOTE*– Carry out the test using low actinic glassware.

*Solvent mixture.* 10 volumes of *hexane*, 7 volumes of *acetone*, 7 volumes of *toluene* and 6 volumes of *ethanol*.

A 0.03 per cent w/v solution of the substance under examination in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 446 nm (2.4.7). Calculate the content of  $C_{40}H_{56}O_2$ , taking 2550 as the specific absorbance at 446 nm.

**Content of Lutein.** Determine by liquid chromatography (2.4.14).

*NOTE*– Carry out the test using low actinic glassware.

*Test solution.* Evaporate 1.0 ml of a 0.03 per cent w/v solution of the substance under examination in the solvent mixture, under a stream of nitrogen to dryness. Dissolve the residue in 1.0 ml of the mobile phase, with the aid of ultrasound.

*Reference solution.* A 0.015 per cent w/v solution of *lutein IPRS* in the mobile phase.

#### Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica (5  $\mu$ m),
- mobile phase: a mixture of 75 volumes of *hexane* and 25 volume of *ethyl acetate*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 446 nm,
- injection volume: 10  $\mu$ l.

The relative retention time with reference to lutein for zeaxanthin is about 1.05.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to lutein and zeaxanthin is not less than 1.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution. The sum of the areas of all the peaks due to lutein is not more than 85.0 per cent, calculated by area normalization.

Calculate the content of lutein  $C_{40}H_{56}O_2$ , using the following expression:

$$\text{Result} = (A_U / A_T) \times T$$

$A_U$  = peak area of lutein

$A_T$  = sum of the areas of all the peaks

$T$  = percentage of total carotenoids as determined in the test for Content of total carotenoids

**Zeaxanthin and other related compounds.** Determine by liquid chromatography (2.4.14), as described under Content of lutein.

Inject the test solution. The area of zeaxanthin peak is not more than 9.0 per cent, calculated by area normalization.

Calculate the content of zeaxanthin, using the following expression.

$$\text{Result} = (B_U / B_T) \times T$$

$B_U$  = peak area of zeaxanthin

$B_T$  = sum of the areas of all the peaks

$T$  = percentage of total carotenoids as determined in the test for Content of total carotenoids

The area of any secondary peak other than lutein and zeaxanthin is not more than 1.0 per cent, calculated by area normalization.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 2.0 per cent.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

**Storage.** Store protected from light, in hermetically oxygen resistant container, at temperature between 8°-15°.

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**Solubility.** Soluble in *ethanol*, *ethyl acetate*, *dichloromethane* and partially soluble in *hexane*.