

Estimation of Carthamin from *Carthamus tinctorius*

ARUNA AHIRE AND K. S. LADDHA*

Department of Pharmaceutical Sciences & Technology,
University of Mumbai Institute of Chemical Technology,
Nathalal Parikh Marg, Matunga, Mumbai-400 019

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A reversed-phase high-performance liquid chromatographic method for the estimation of carthamin in *Carthamus tinctorius* is described. The aim of this work is to provide a simple and accurate method of standardization of *C. tinctorius* flower petals, extracts and formulations made from them for carthamin.

In recent years, natural colourants have been re-examined from the point of safety, especially in the field of food and cosmetic industry¹. Carthamin, the red colouring principle of the petals of *Carthamus tinctorius* Linn., (Safflower, Family: Compositae) has long been used in cosmetics, food, beverages, soft drinks, fruit jams and textiles. Carthamin belongs to the class of quinochalcone C-glycoside (fig. 1) and it is claimed as hypolipidemic, hypocholesteremic and antiobesity agent². Therefore, estimation of carthamin would be an important parameter for the quality control of *Carthamus tinctorius* petals or the extracts and formulations made from it. This paper deals with the development of simple, precise and accurate method to estimate carthamin by reversed-phase high-performance liquid chromatography in dried petals of *C. tinctorius*.

Different safflower varieties (DSH-129, *Sharda*, JSI-7, JSI-97, JSI-103 and *Nari-6*) cultivated at Parbhani region of Maharashtra state were obtained for analysis. Carthamin was supplied by Safflower Institute of Tianjin, China. All solvents used were of HPLC grade.

A Jasco HPLC system consisting of a Jasco PU-1580 Intelligent and Jasco PU-2080 Plus Intelligent pumps, Jasco UV-1575 intelligent UV/Vis detector, Rheodyne 7725 loop injector, Lichrospher 100 RP-18e (250 mmx4.6 mm I.D.), 5 μ m column and Borwin software version 1.50 was used. The content of carthamin in supplied sample (Tianjin, China)

For correspondence

E-mail: ksladdha@yahoo.co.in

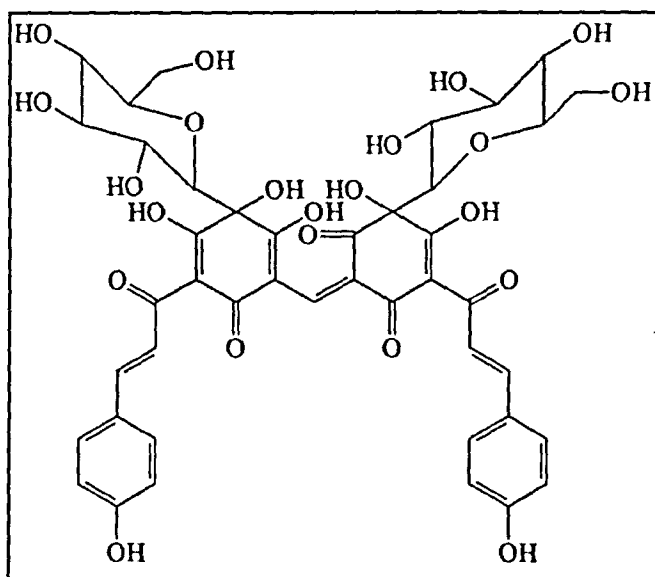


Fig. 1: Structure of carthamin

was determined by $E_{1cm}^{1\%}$ value (992) at 530 nm in dimethyl formamide³⁻⁵. The sample was found to contain 15.15 % w/w of carthamin. Standard stock solution of carthamin was prepared to get 15 μ g/ml solution and this solution was suitably diluted with mobile phase for the calibration graph. For the separation of carthamin, isocratic elution was used with methanol-water (45:55) at the flow rate of 1 ml/min. The column temperature was ambient and the detection was performed at 520 nm. The chromatogram was obtained by in-

jecting 20 µl of each solution into the HPLC system. Intra and inter day assay precision was determined by analyzing solution in single day and on three consecutive days.

The powdered petals (# 36/60) were extracted with each 20 ml portion of 60 % acetone for 5 times at room temperature, filtrates were combined and volume was made to 100 ml. From this suitable dilution (1:10 to 1:20) was made with mobile phase and subjected to analysis. The results are shown in Table 1. Recovery experiment was conducted by adding known amount of carthamin to the previously analyzed sample of *C. tinctorius* petals extract.

TABLE 1: CONTENT OF CARTHAMIN IN DIFFERENT VARIETIES OF *CARTHAMUS TINCTORIUS*

Variety	Carthamin % w/w (Mean±S.D.)
DSH-129	0.040±0.002
Sharda	0.048±0.002
JSI-7	0.063±0.004
JSI-103	0.085±0.003
JSI-97	0.090±0.006
Nari-6	0.206±0.006

All the values are the averages of three determinations, SD: Standard deviation.

The retention time of the carthamin in RP-HPLC was 6.00 (±0.10) min (fig. 2). Limit of detection and limit of quantification was found to be 45 ng/ml and 60 ng/ml, respectively. Calibration curve was linear in the range of 60 to 420 ng/ml. Further a correlation coefficient of 0.9978 indicates good linearity between concentration and area. Percent coefficient of variation for the intra and inter day assay precision was 0.039 and 0.032, respectively. The extracts of different varieties of *C. tinctorius* showed no significant peaks at the retention time other than the retention time of carthamin which indicates co-extractives do not interfere in the estimation, using RP-HPLC method (fig. 2). The method allows reliable estimation of carthamin. The result of recovery study, 101.5 % indicate the method is accurate. Thus the method is simple and convenient to carry out routine analysis.

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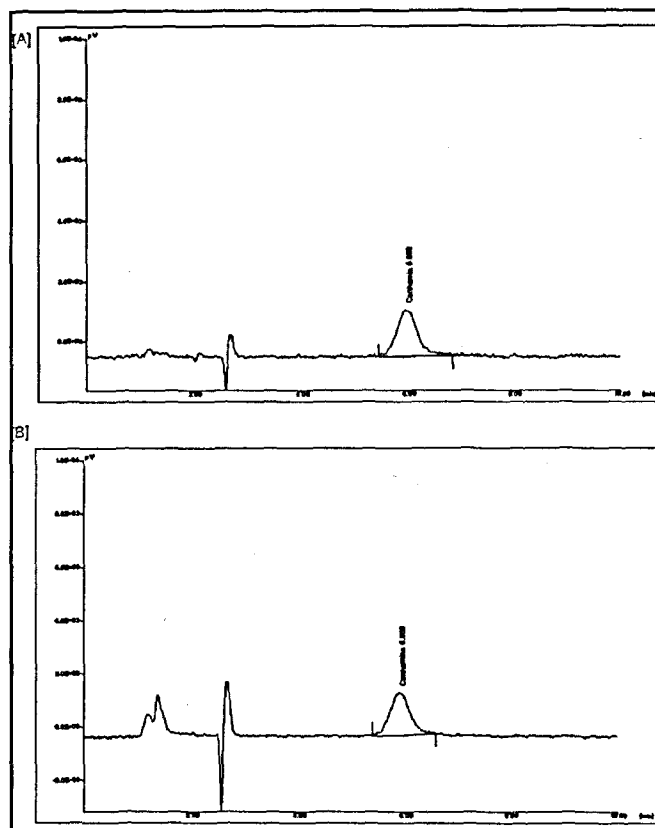


Fig. 2: HPLC chromatogram of carthamin and *Carthamus tinctorius* petals extract

[A] HPLC chromatogram of carthamin [B] HPLC chromatogram of *Carthamus tinctorius* petals extract under identical conditions.

Marathwada Agricultural University, Parbhani, for the supply of safflower petals.

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