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Development and Validation of HPLC Method for Determination of Crocetin, a constituent of Saffron, in Human Serum Samples

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ABSTRACT

Objective(s): The present study reports the development and validation of a sensitive and rapid extraction method beside high performance liquid chromatographic method for the determination of crocetin in human serum.

Materials and Methods: The HPLC method was carried out by using a C18 reversed-phase column and a mobile phase composed of methanol/water/acetic acid (85:14.5:0.5 v/v/v) at the flow rate of 0.8 ml/min. The UV detector was set at 423 nm and 13-cis retinoic acid was used as the internal standard. Serum samples were pretreated with solid-phase extraction using Bond Elut C_{18} (200mg) cartridges or with direct precipitation using acetonitrile.

Results: The calibration curves were linear over the range of 0.05-1.25 µg/ml for direct precipitation method and 0.5-5 µg/ml for solid-phase extraction. The mean recoveries of crocetin over a concentration range of 0.05-5 µg/ml serum for direct precipitation method and 0.5-5 µg/ml for solid-phase extraction were above 70 % and 60 %, respectively. The intraday coefficients of variation were 0.37- 2.6% for direct precipitation method and 0.64 - 5.43% for solid-phase extraction. The inter day coefficients of variation were 1.69 – 6.03% for direct precipitation method and 5.13-12.74% for solid-phase extraction, respectively. The lower limit of quantification for crocetin was 0.05 µg/ml for direct precipitation method and 0.5 µg/ml for solid-phase extraction.

Conclusion: The validated direct precipitation method for HPLC satisfied all of the criteria that were necessary for a bioanalytical method and could reliably quantitate crocetin in human serum for future clinical pharmacokinetic study.

► ***Keywords:*** Crocetin, *Crocus sativus*, Direct precipitation, High performance liquid chromatography, Human serum samples, Saffron, Solid phase extraction

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