

# Quantitative determination of crocin-1, crocin-2, picrocrocin and safranal in saffron-based food supplements

## Instruments and conditions

Method: U(H)PLC/UV/MS (ultra-high performance liquid chromatography with UV and MS detection).  
System: Acquity UPLC H-Class (Waters, Milford, MA, USA)  
Detector 1: PDA: Acquity UPLC PDA e, -range: 200-800 nm  
Detector 2: QDa: Acquity UPLC QDa, ESI, single-quadrupole, m/z-range: 100-1200 Da  
Temperature: 40 °C  
Flow rate: 0.55 mL/min  
Inj. volume: 2 µL  
Stat. phase: Acquity UPLC HSS T3, 1.8 µm, 2.1 mm x 100 mm  
Mobile phase: Gradient elution

## Gradient elution

time [min]	A H <sub>2</sub> O + 0.1 % formic acid	B Acetonitrile + 0.1 % formic acid
0	98	2
0.5	98	2
6	50	50
7	0	100
8	0	100
8.2	98	2
10	98	2

## Sample preparation

The samples were weighed into a conical centrifuge tube (15mL Falcon tube) and 10 mL extraction solvent (water-methanol 8:2, v/v) was added. The suspension was extracted in an ultrasonic bath for 5 min and then centrifuged at 8000 rpm for 3 min. The supernatant was transferred to an HPLC sample vial and measured.

For samples A1-3 the contents of 2 capsules each were taken, while for samples B1-5 and C only one capsule was weighed. Thus, a dosage of 30 mg saffron extract of the samples (28 mg for sample C) was examined. At the same time, 30 mg of saffron (stigmas) were mortared with sea sand and extracted in an analogous manner as a reference.

## Quantification

Quantification was performed relative to four external standards: crocin-1, crocin-2, picrocrocin and safranal. Linearity in the working range was shown by calibration curves. In the case of crocin-1, recovery and accuracy were checked using the standard addition procedure. A determination of the precision and repeatability of the method was also made.

## Structural formulae and example chromatograms

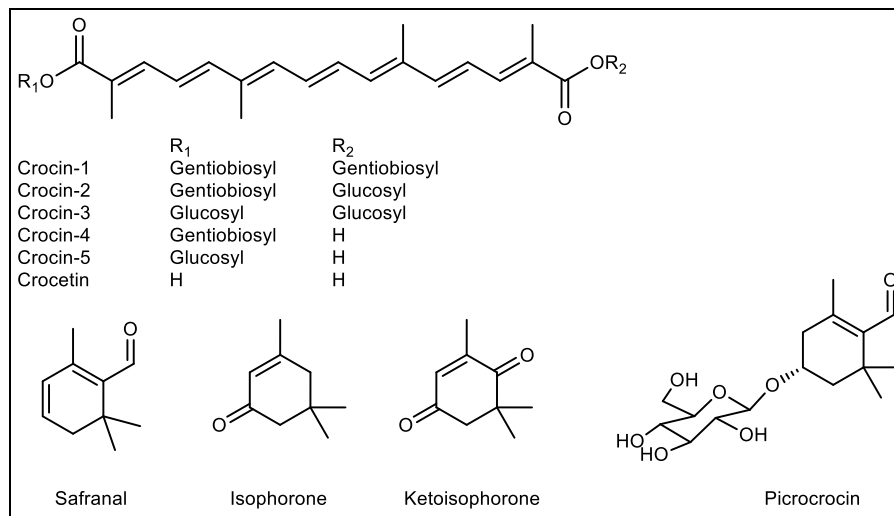


Fig. 1 Structural formulae of some saffron constituents

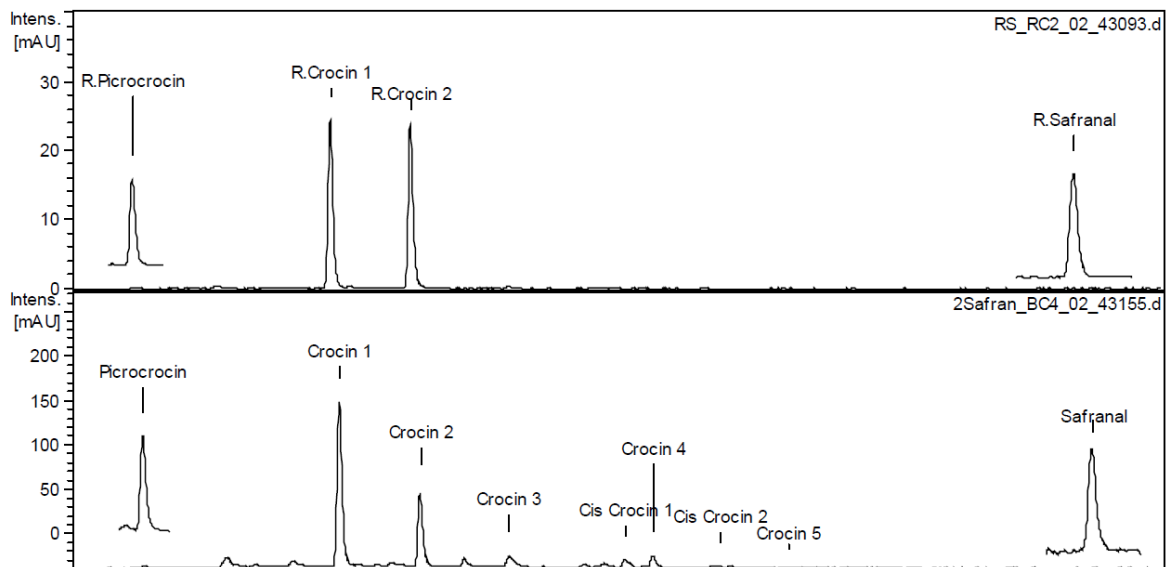


Fig. 1 Example chromatograms (440 nm). Top: reference substances, bottom: authentic saffron extract. The chromatograms at 256 nm (picrocrocin) and 330 nm (safranal) are only shown in section for the sake of clarity.

The method and Fig. 2 are taken from the following Master's thesis:

### *Investigation on the quality of saffron-based food supplements*

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