Extraction of Saffron Crocin as a Natural Pharmaceutical Source with Crystallization Method

Sahebeh Sedaghati, Fatemeh Abbaszadeh
Bahraman Saffron Company, R&D Department, Mashhad, Iran
Email: s.sedaghati70@yahoo.com, Abaszadeh_f@ymail.com

***Abstract*—separation of crocin as anti-cancer and antioxidant ingredient would be useful commercially and clinically. In this research with crystallization method, saffron Crocin was extracted. Ethanol 80% and acetone was chosen as the best extraction solvent. Crystallization and purification process was performed in two steps in zero and -5c° degree. In first step, saffron Crocin was extracted with ethanol and after keeping in -5c° for 23 days obtained Crystals were separated. Obtained Crocin crystals from the first step had low purity and the pure crystals were yielded during the second crystallization. Extraction and purity of Crocin. Crystals were studied by UV-visible spectrophotometry and Fourier transform spectrometry and High Performance Liquid Chromatogram analysis compared to Crocin Sigma-Aldrich. Results shows that the extraction intensity and purity of the obtained Crocins were significantly higher (28.32 times). Measurement of color showed the color strength was more than sigma-Aldrich about 7.5 time.**

The results of this research showed that purchased Crocin according to the chromatograms is not pure and some unknown impurity were seen. Besides, Chromatogram spectra's shows that obtained Crocin crystals were in higher purity than purchased one.

This information illustrated that this crocin because of high purity can be used as a reliable and valid standard.

***Index Terms*—chromatogram, crocin, ethanol, extraction, purity, saffron**

I. Introduction

Saffron with Scientific name (*Crocus Sativus*), is one of the most expensive spice in the world. Saffron plant is mainly cultivation in countries such as Iran, Egypt, France, Turkey, Italy and Spain [1]. In average annually 205 ton saffron is producing and Iran with 47000 cultivate Hectares of saffron plant is allocated about 80% of this product to itself. Saffron is using in various industries such as food, pharmaceutical and textile. In food industry mainly use as food additive for tasting, flavoring and coloring. In textile industry for creating red color in silk fabrics and in pharmaceutical industry, it is used to prevent, control and treatment of diseases [2], [3]. Saffron main component is Crocin ($C_{44}H_{64}O_{24}$) color factor, Picrocrocin ($C_{16}H_{26}O_7$) the bitter taste of saffron factor and Safranal ($C_{10}H_{14}O$) Flavor factor that shown in Table I [3].

In fact Crocins are a group of water soluble carbohydrate compounds in saffron stigmas that they composed of mono and di glycosylic esters of polyene dicarboxylic acid (Hadizadeh, 2014). Crocin and Crocetin esters has multiple biological properties. It has been reported that crocin has many medical properties such as antioxidant, anticancer, anticoagulant, lipid regulator, neuronal protector, anti-tumor, and so on [5]-[7].

Crocin has several glycosylic esters that on which about 6 of them detected in saffron. Crosin Analogues that include Crosin 1 to 4 is mainly from saffron Trance-Crocetin glycosides that from them Trance-Crocetin 3 and 4 has the greatest abundant in saffron [8]. For Quantitative and Qualitative Analysis of Derivatives Extracted from Saffron we can use various methods such as UV [1]Spectrophotometry, NMR[2], TLC[3], GC-MS[4] and HPLC[5] [9], [10].

Besides there are different analytical methods for extracting and purifying Crocin and its derivatives like Extraction with Ultrasound Waves, Supercritical CO2 and Extraction by Crystallization [4]. The quantity and quantity of the extracted crystals are depends on the extraction method, drying method and the storage condition.

The Methods of extracting saffron metabolites can be optimized by Solvent type, temperature, light and stirring time process [9].

Different solvents such as ethanol, water, diethyl ether and acetone are used for extracting Crocin [4], [9], [10]. Extensive studies have been conducted on chemical and quantitative analysis of Crocin but focus on Crocin extraction and purification methods has been studied less. Extraction with solvent is one of the simplest and affordable ways of obtaining Crocin with higher purity.

The purpose of this research is to focus on the production, extraction and purification of saffron Crocin with crystallization method.

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[1] Ultraviolet/Visible
[2] Nuclear Magnetic Resonance
[3] Thin layer chromatogram
[4] Gas chromatography-Mass spectrometry
[5] High Performance Liquid Chromatography

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II. Material and Method

Crocin sample with batch number: 17304 was purchased from Sigma-Aldrich Company (Tehran- Iran). Saffron stigmas were provided from Bahraman Saffron CO. (Mashhad-Iran). Ethanol and acetone 96% obtained from Pars Alcohol CO. (Tehran- Iran). All the other solvents used in chromatography analysis has HPLC grade and purchased from Merck CO. (Germany).

A. Extraction of Crocins from Saffron Stigmas

10 gr of top Bahraman saffron grade 1 was grinded with Laboratory mill (Germany-No. A10 IKA) in to powder and sieved with mesh no: 60. Suspended saffron powder with ethanol 80% in 0 ℃ and stirring for 2 min. after centrifugation at 3000 RPM for 10 min a solution of centrifugal suspension was extracted. Then, 25 milliliter of ethanol 80% was added to sediment in the same situation (0 ℃ and stirring for 2 min) and centrifuged.

This process was repeated several time and 200 milliliter ethanol 80% was used in total for extracting Crosin from 10 gr saffron. Eventually, sediments color were changed to orange to yellow. Extracted liquid from above steps were kept in -5 ℃ for 23 days in sealed dark containers. Obtained Crocin crystals were washed with acetone for separating from solution. The obtained crystals were dissolved in 120 milliliter ethanol 80% for the second time and kept in the same situation for extra days for recrystallization. The final amount of yielded crystals were separated and its efficiency was calculated (1 gr).

B. Preparation Sample for Analysis

Extracted samples were stored in a sealed dark container in 2 to 5 ℃ before testing.

C. Spectrophotometry

The absorbance rank of produced Crocin in Bahraman saffron laboratory and sigma Crocin was measured under the standard (259-2) at 440 nm wavelength [11]. Color strength was calculated by following equations, as in (1):

$$CS = (A/W)*h*100 \quad (1)$$

That CS is sample color strength, A is absorbance rank, W is sample weight and H was Sample index that calculated with following equations, as in (2)

$$H = [100/ (100- \text{moisture})] \quad (2)$$

D. HPLC Analysis

All the chromatogram analysis were done under the RP-HPLC with methanol-water solvent, with 1ml/min at 440 nm and 250 nm wavelengths.

III. Results

A. Spectrophotometry

The samples UV-VIS absorbance rank was measured and the color strength was calculated. (Table I) results shows that, color strength of the produced samples were much higher than the market samples.

Results shows that the UV absorbance rank of the produced Crocin crystals in 440nm wavelength were 7.5 times higher than the sigma samples. Since absorption at this wavelength represents the density and intensity of the samples, it can be concluded that the produced crystals had a higher intensity. Absorbance rank in 250nm wavelength that showed the Picrocrocin rank, was 3.4 time higher than sigma sample. This result showed that the amount of extracted material in the crystals were higher than the sigma sample.

TABLE I. Absorbance Rank and the Samples Color Measuring in 440, 250nm

Sample	440nm		250nm	
	A	Color measuring	A	Color measuring
Sigma crocin	0.062	134.664	0.032	69.504
Crystal produced crocin	0.47	1020.89	0.109	236.748

B. High Performance Liquid Chromatogram

Both samples chromatogram in 440nm wavelength was shown (Fig. 1). The results of this chromatogram showed the presence of some types of Crocin in both samples. The total amount of Crocin in produced crystals were 28.32 times higher than the market ones (Table II). According to Table II highest level was belongs to retention time 13.050 that contains 66% of the area under curve. The calibration curve of the test was also shown in (Fig. 2). These results indicated that the total amount of contained Crocin in the purchased samples was less purified than the total contained Crocin in the produced crystals.

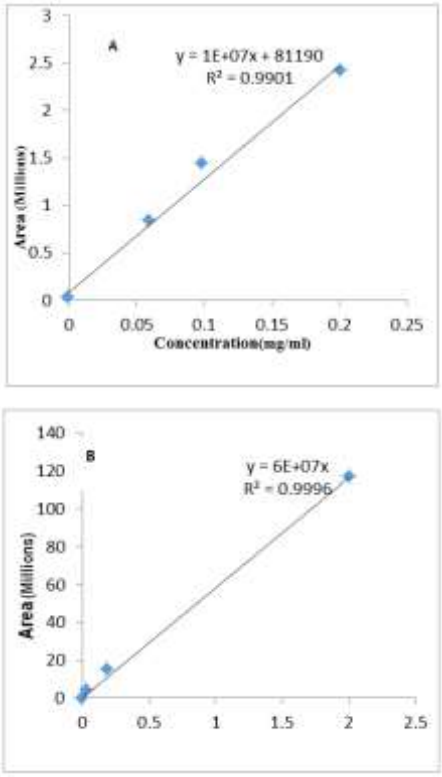

Figure 1. Calibration curves sample at 440nm. A: Sigma aldrich crocin B:crystal crocins.

TABLE II. ETENTION TIME, AREA AND HEIGHT OF CRYSTAL CROCIN AT 440 NM

Retention Time	Area	Area %	Height	Height %
12.000	224232	0.48	18060	0.58
12.583	156075	0.33	12477	0.40
13.050	31248849	66.32	2086947	66.69
13.833	11919527	25.30	798586	25.52
14.567	7264	0.02	1706	0.05
15.733	492242	1.04	28969	0.93
16.350	3071822	6.52	182359	5.83
total	47120011	100.0	3129104	100.0

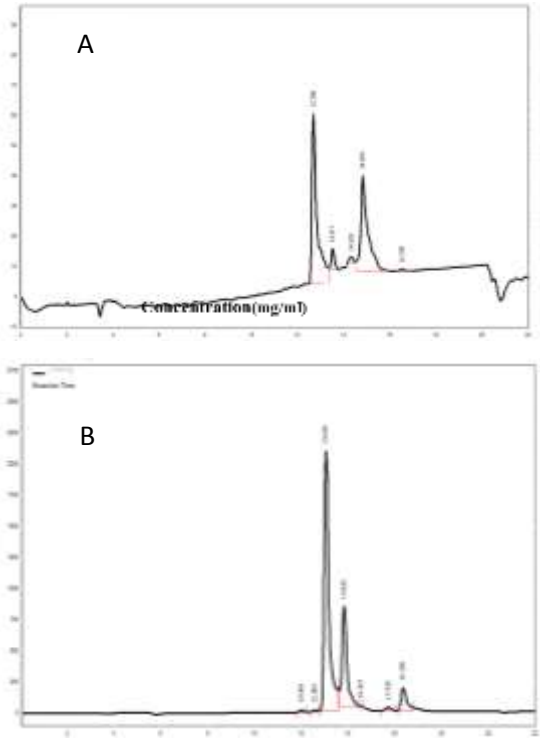

Figure 2. Samples chromatogram (0.1mgr/mlitr) at 440nm. A:Sigma aldrich crocin B:crystal crocins.

An impurity was observed at 250nm wavelength of purchased samples chromatogram (Time = 11), but this impurity was not detected in produced Crocin crystals. Also Picocrocin peaks in both samples were presented before the Crocin peaks (time = 13), and much higher in produced Crocin than the purchased ones (Fig. 3). Generally, in Sigma crocin the area under curve (AUC) of the impurity, picrocrocin and total crocin was 8.9, 3.1 and 32.02% and for crocin crystal, at this wavelength was 0, 60.29 and 7.11%.

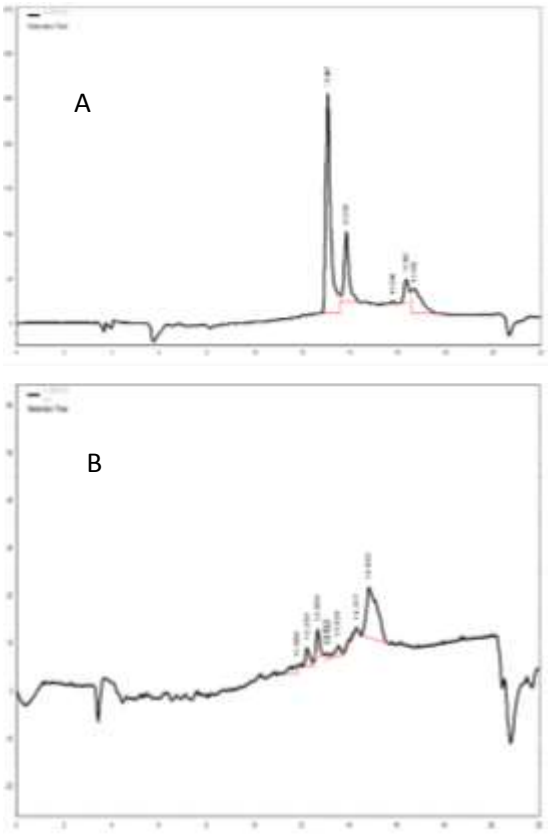

Figure 3. Samples chromatogram (0.1mgr/mlitr) at 250nm. A:Sigma aldrich crocin B:crystal crocins.

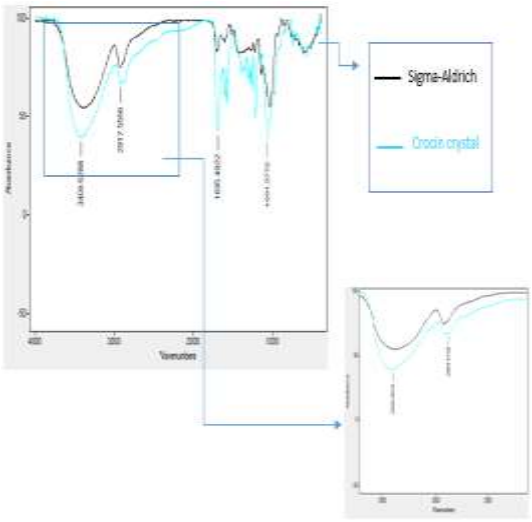

Figure 4. Fourier transform infrared spectrometry of crocin crystal.

C. Fourier Transform Infrared Spectrometry

The infrared test is used to determine the functional groups and the intensity of its presence in the substance. The spectroscopic studies were shown in Fig. 4. The intensity of the Crocin Crystals factors were much higher than the market Crocins. These results showed that the intensity of Crocin, in other words, the purity of the sample was higher than the sigma.

IV. Conclusion

All the obtained datas were showed that the amount of Crocin present in the samples were significantly higher than the Sigma Crosin, which could be used as a reliable standard. In this study, we achieve to develop a high purity Crocin with a simple and inexpensive method. Now a days due to its unique properties, advanced countries were using from this healing substance for treating diseases such as MS, Cancer, Tumors, and Depression.

It is hoped that according to Density and purity of prepared substance, this material could be used in various pharmaceutical and food industries.

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Sahebeh Sedaghati is the MSc graduated from the Ferdowsi University of Mashhad (www.um.ac.ir) and R&D expert in Bahraman Company (www.Bahramansaffron.ir)
Her activities is: Extraction bioactive compound of saffron; Purification of crocin and safranal ; Edible composite film based on gum and protein; Edible film from extruded protein.

Fatemeh Abbaszadeh is the MSc student from Azad University of Mashhad (www.mshdiau.ac.ir) and R&D expert in Bahraman Company (www.Bahramansaffron.ir)
Her activities is: Extraction bioactive compound of saffron; Purification of crocin and safranal .