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Direct Analysis of Sunset Yellow in Commercial Saffron Using Gradual Solvatochromic Effect Followed by Rank Annihilation Factor Analysis (RAFA)

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ABSTRACT

In this study, a new spectrophotometric method for the determination of sunset yellow in adulterated Saffron without pre-separation has been developed.It was found that a second order spectra data matrix of Sunset yellowconstructed from thesolvent components gradual change–visible absorption spectra can be expressed as thecombination of two bilinear data matrices. With the bilinear model, the second order spectra data of mixtures containing Sunset yellow coexist with interferents (red pigments of Saffron) could be analyzed using the second order calibration algorithms.The algorithm used here was rank annihilation factor analysis.In the method investigated here, the components of the solvent were changed gradually byadding 1,4-Dioxane into water,subsequently, the absorption spectra of Sunset yellow and Saffron samples in aseries of 1,4-Dioxane –water mixed solvents, wererecorded. Thus, the concentration of Sunset yellow in a gray system could be determined fromthe spectra matrices using second order calibration algorithms.This method is simple, convenient and dependable. The method has been successfully used for analysis of Sunset yellow in adulterated Saffron with satisfactory results.

Keywords: Sunset yellow; Adulterated Saffron; Solvatochromism; Rank annihilation factor analysis; Water–1, 4-Dioxane mixed solvent

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INTRODUCTION

Saffron, is produced from dried stigmas of “Crocus Sativus L” [1]. It is known as the most expensive spice in the in the world. Addition of its traditional value as a food additive, Saffron has been used in traditional medicine for a long time. The modern studies have shown its potential as an anti-cancer agent [2, 3] and demonstrate the carotenoid compounds in the Saffron could inhibit growth of tumors [4].

Because, Saffron is a rather high value plant, it is become frequently adulterated. Typical methods of Saffron adulteration include mixing of extraneous substances like beets, pomegranate fibers and red synthetic dyed such as Sunset yellow, Tartrazine, Ponceau4R, Methyl orange and Erythrosine. The most common way of adulteration is the mixing of artificial colorants. These practicesperfect the appearance of the dried stigmas and increase the coloring strength [4, 5].

Quality control of commercial Saffron is resolved by specifications recommended by the ISO 3632 [6].

One of the main purposes of this procedure is the detection of added colors in the presence of "Crocin", Saffron natural color. The absence of these synthetic colors has to be indicated by TLC or/and ion-pair HPLC. This procedure has some inconvenience and is very time consuming involved sample preparation of Saffron. In addition, it involves the using of organic solvents that are environmentally harmful.

The method of Saffron quality control that can be offered is UV–Vis spectrophotometry because it is quick, easy and low cost.

Unfortunately, this technique is non-specific and unable to adequately identify trueand false Saffron due to overlapping of the signal and thus unable to provide a quality category on the international market [5,7].

In most cases, these problems are associated with the removal of the undesired components [5] without affecting the concentration and prototype of the analytes, but these separation methods have some disadvantages such as they arehard, expensive and in some cases aren't available [8].

So, it is vital to develop some alternative spectrophotometricmethods without pre-separation for detection of false Saffron.

Multivariate and multi-way spectroscopic methods in combination with chemometrics data analyses can be used for quality control of food [4, 9].

For the quantitative analysis of target analytes in mixtures containing unknown interferents (gray

systems), without pre-separation, second-order calibration algorithms can be utilized[10]. The most of these methods, such as parallel factor analysis (PARAFAC) [11–14], rank annihilation factor analysis (RAFA) [15,16], generalized rank annihilation method (GRA)[17,18], residual bilinearisation (RBL) [19,20] and unfolded partial least-squares/residual bilinearisation (U-PLS/RBL)[21,22], are based on the rank analysis for the second-order data matrices of the system that are produced by proper manners. For using these algorithms, the second-order data matrices should be able to be bilinearly analyzed [23].

The absorption spectra of analytes are often affected by the components of solvent, in the other words solvent polarity. It is referred as "solvatochromism". Along with the change of solvent components, their absorption peaks usually red-shift (bathochromic shift) or blue-shift (hypsochromic shift) and the absorbance values also change accordingly. The sign of the solvatochromism depends on the difference in dipole moment between the ground and excited states of the chromophore [24].

In general, for different analytes, the effects of solvent components on their absorption spectra are different. Using the difference of absorbance values of the analytes in different solvents, some second-order spectra data could be produced and could be used to determine the analytes in mixtures containing unknown interferents by proper chemometrics technique. Now, there are a few reports concerning the determination of the target analytes in mixtures containing unknown (or uncalibrated) interferents using the second-order spectra data produced with this manner [23, 25-26].

In the present study, it was found that the visible absorption spectra of Sunset yellow in pure water were different from the spectra in pure 1, 4-Dioxane solvent. In addition, it was also realized that an absorption spectrum of Sunset yellow in a water–1, 4-Dioxane mixed solvent could be expressed as a linear combination of a spectrum of Sunset yellow in pure water with a spectrum of Sunset yellow in pure 1, 4-Dioxane. In the other hand, the second order data matrix of Sunset yellow produced from the spectra of Sunset yellow in a series of water–1, 4-Dioxane mixed solvents with various 1, 4-Dioxane volume fractions could be expressed as the combination of two bilinear data matrices. Based on this discovery, herein a novel technique for the determination of Sunset yellow in gray mixtures containing uncalibrated interfernts has been investigated from the second order data of solvent component gradual change–visible spectra using RAFA algorithms. This method is the first analytical method that uses the change of solvent components to analysis of Sunset yellow in adulterated Saffron.

In the proposed method, complicated operation of pre-separating mixtures is not necessary, the way of producing second order data is direct and dependable, and the apparatus used is common.

MATERIALS AND METHODS

2.1. Apparatus and software

Visible absorbance spectra were collected using Perkin-Elmer Lambda 45 spectrophotometer equipped with a 1 cm path length quartz cell. The spectrophotometer was interfaced to a personal computer. The recorded spectra were digitized with one data point per nanometer. The spectral wavelength range was 320–600 nm. A Metrohm 744 pH-meter furnished with a combined glass-saturated calomel electrode was used for pH measurements. All calculations were done using MATLAB® 7.6[27].The m-file for performing of RAFA was downloaded from http://www.iasbs.ir/chemistry/chemometrics/history/5th, thanks to H. Abdollahi.

2.2. Reagents and solutions

All chemicals used in the experiments were of analytical grade and used without further purification. All solutions were prepared with doubly distilled water. Stock solutions of 1000

mg L^{-1} of Sunsetyellow FCF(Sigma) was prepared by direct dissolution of 0.1000 g of the compound in 100.0 mL doubly distilled water and was stored in plastics amber bottles at 5°C and protected from light.

Working solutions were prepared by appropriate dilution of the stock solution.

2.3.Preparing the adulterated Saffron

Dried stigmas of safe Saffron samples were purchased directly from a farm in Mashhad, Iran.

Saffron powder (0.1001 g) was weighed into a glass centrifuge tube to which water (10 ml)was added. The mixture was vortexed (1 min), kept in the dark (10 min), agitated again (1 min) and finally centrifuged (6000 rpm, 15 min). The supernatant was passed through a Whatman filter of 0.45µm pore size and diluted in a 100.0 mL volumetric flask [28]. Adulterated Saffron samples were prepared by spiking desired amounts of Sunset yellow into appropriate diluted Saffron solutions.

2.4. Procedure

Two milliliters of standard solution containing suitable amount of Sunset yellow or sample solution was added into the cell. Then the solution was stirred and 1, 4-Dioxane was added gradually. After every 25µL of 1, 4-Dioxane added, spectrum scanning was carried out in the wavelength range of 320–600 nm. The total added volume of ethanol was 1 mL.

RESULTS AND DISCUSSION

3.1. Absorption spectra of Sunset yellow in 1, 4-Dioxane and water

Typical normalized absorption spectra of Sunset yellow (Scheme 1) in pure 1,4-Dioxane and pure water are shown in Fig. 1. From Fig. 1, it can be seen that the spectral profile of Sunset yellow in 1,4-Dioxane is different from that in water. When the solvent is changed from water to 1, 4-Dioxane, red-shift of absorption peak of Sunset yellow occurs slightly and the absorbance values also change correspondingly.

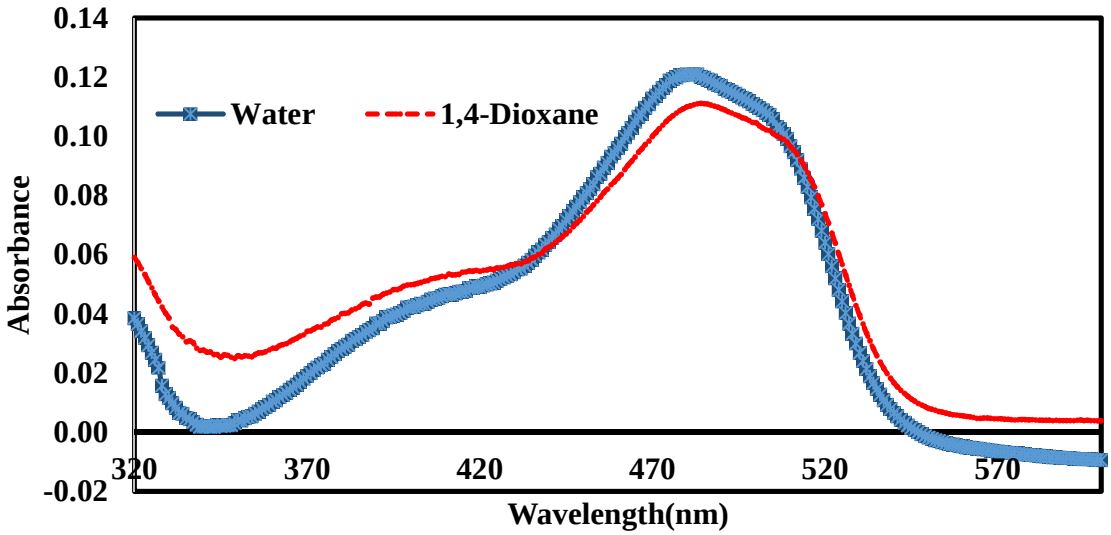
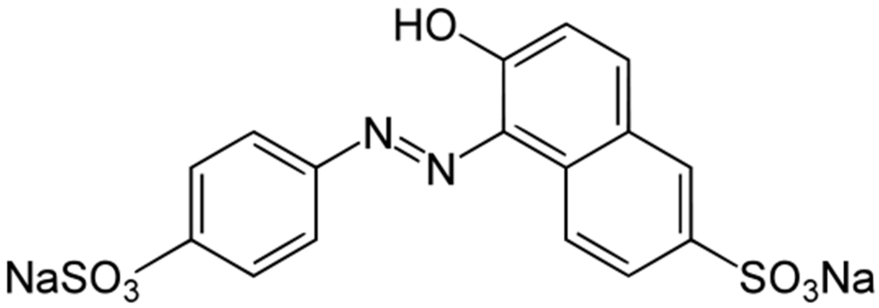


Fig. 1. Normalized Absorption spectra of Sunset Yellow in pure water and 1, 4-Dioxane



Scheme 1 – Chemical structure of Sunset yellow

3.2. Evolution of bilinearity in second order absorption spectra matrix of Sunset yellow

For a certain concentration of Sunset yellow, when absorption spectra at *n (=40)* added volume point of 1, 4-Dioxane were scanned and the absorbance values at *p (=280; 320-600nm)* wavelength points were recorded, data matrix could be shown as follows:

$$\mathbf{A}_{np} = \left(a_1^t, a_2^t, \ldots, a_i^t, \ldots, a_n^t\right) \qquad \text{Eq. (1)}$$

Where $a_i = (a_{i1}, a_{i1}, \ldots, a_{ij} \ldots, a_{ip})$ is the absorption spectrum vector at added 1,4-Dioxane volume, a_{ij} is the absorbance value at wavelength point *j*, superscript *t* denotes the transpose of a matrix or vector.

Singular value decomposition was used to analyze matrix **A**(in different concentration of Sunset yellow). The results show that the number of significant factors of matrix A is only **two**, which means that the rank of **A** is two (Fig. 2) or means that Sunset yellow exists in water- 1,4-Dioxane mixed solvents with two species and the absorption spectra of the two species are different. Thus, a spectrum of Sunset yellow in a mixed solvent can be considered as the result of the linear combination of a spectrum of one species with a spectrum of another species.

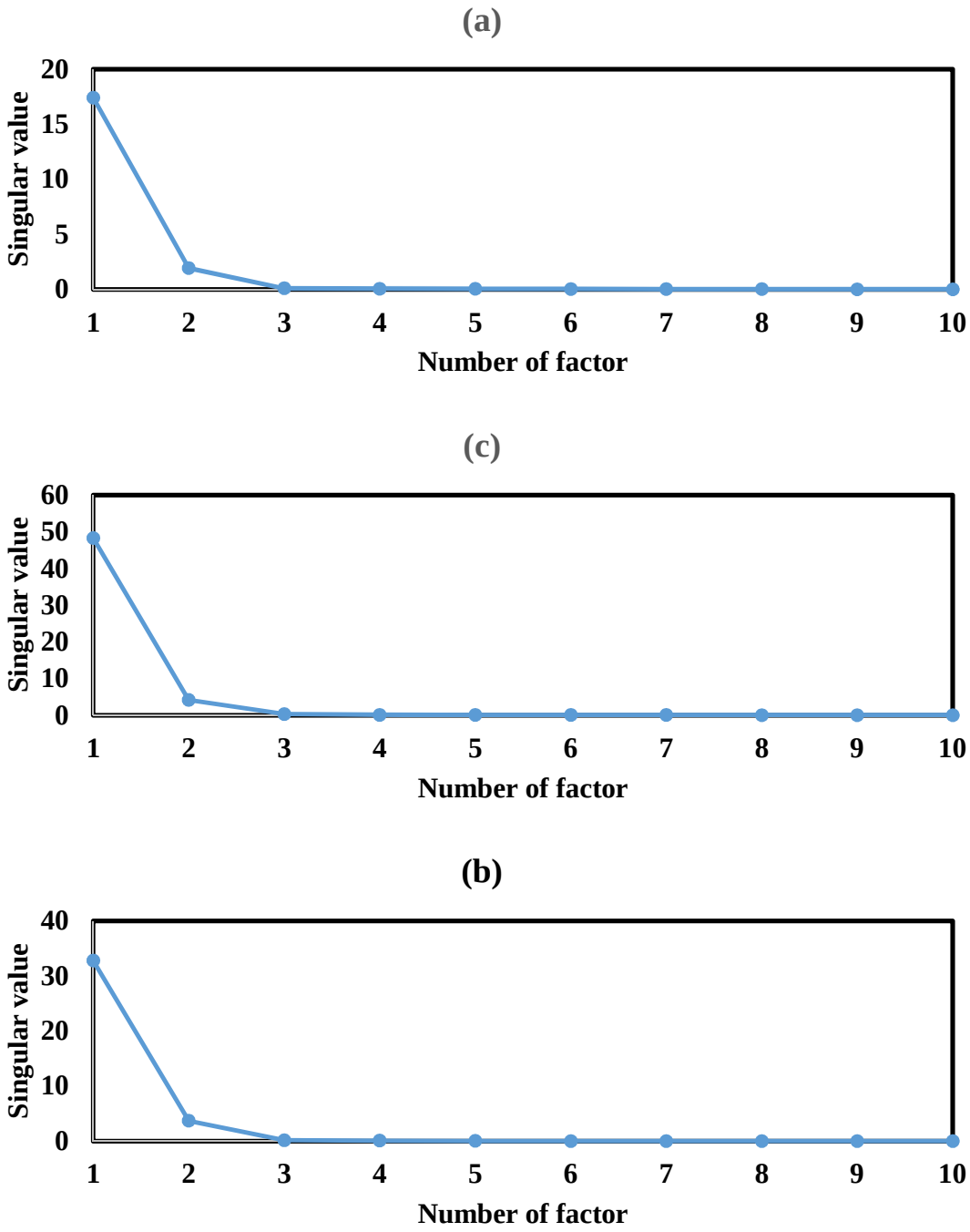


Fig.2. Calculated singular values for second order spectra data matrix of Sunset yellow.
Sunset yellow concentration (a):9; (b):18.1 and (c):27.1 mg L^{-1}.

3.3. Second order calibration algorithm: Rank annihilation factor analysis

To quantitative analysis of the desired analyte, in complex matrix with unknown interference, using second order data, it is very important to select proper chemometrics technique according to the character of the data. As it was known, Sunset yellow has two absorption spectra in mixed solvent. The absorption spectra of Saffron in pure water and 1,4-Dioxane, are shown in Fig. 3.It display that other species related to natural red pigments of saffron have visible spectra too and they overlap with the spectra of Sunset yellow in pure water or 1,4-Dioxane. Thus, it can be

resulted that there are linear dependencies in the second order data, or the spectral matrices are rank deficient. Therefore, some second order calibration algorithms cannot be applied to linear dependent data. RAFA can be used to analyze the second order data matrices whether the matrices are rank deficient or not and its algorithms is very simple and direct.

At first, let us consider **A** as a data matrix obtained by spectrophotometric/solvent titration that can be decomposed into two concentration and spectral matrices **C** and **S**, respectively.

$$\mathbf{A} = \mathbf{C}\mathbf{S}^{\mathbf{t}} + \mathbf{E} \qquad \text{Eq. (2)}$$

Matrix **A** is the spectral data matrix containing rows of visible spectra recorded during the titration experiment. The columns of the **C** matrix are the pure solvent -dependent concentration profiles of the modeled components and the $\mathbf{S}^{t}$ matrix are their related pure spectra and **E** is the matrix of residuals which not explained by the model and includes any other components in unknown matrix. When the sample solutions consist of just one component, two chemical components, $A_{solvent1}$ and $A_{solvent2}$, are expected. If both of them were active in visible region, rank 2 is obtained for this data matrix. But, if in addition of analyte, other components are present in the sample, some other equilibria should be considered and in this case and the calculated rank of the system differs from the expected values.

The rank analysis on the data matrix **A** can be done by singular value decomposition (SVD). Chemical components give rise to larger singular values than noise or instrument contributions. Therefore, the chemical rank can be estimated by the number of singular values larger than singular values associated with noise. In closed system, containing analyte and other interfernts, the number of significant components calculated by SVD is lower than the true number of spectroscopically absorbing species that are expected based on chemical information. This phenomenon is known as rank deficiency and completely hinders the correct resolution of the respective data matrix[29,30].

"In RAFA algorithm, the main matrix equation analyzed can be expressed as follows:

$$\mathbf{A_x} = \beta\mathbf{A_s} + \mathbf{R} + \mathbf{E} \qquad \text{Eq. (3)}$$

Where $\mathbf{A_s}$ is the second order spectra data matrix of a Sunset yellow standard solution, $\mathbf{A_x}$is the second order spectra data matrix of a mixture sample of Sunset yellow mixed with pigments in Saffron components. **R** is the background matrix, **E** is the remnant error matrix and β is the regression coefficient which is the ratio of c_x/c_s (where c_x and c_s are the concentrations of Sunset yellow in mixture sample and in standard solution, respectively)" [25].

The process of RAFA algorithm can be described as follows: let different values in a rational range be assigned to β, then execute principal component analysis for the matrix $\mathbf{A_x}$ $\beta\mathbf{A_s}$ with each βvalue, extract n principal components to constitute new matrix **R***and calculate the eigenvalues g_i of **R*.** The optimized solution can be reached by decomposing matrix **R*** to the extent that RSD of the residual matrix reaches a minimum. The RSD is a measure of the lack of fit of a principal component modeled to a data set. The RSD is defined as below [30]:

$$\mathrm{RSD(n)} = \left(\sum_{i=n+1}^{c} \frac{g_i}{[n(c-1)]} \right)^{1/2} \qquad \text{Eq. (4)}$$

Where $g_i$is the eigenvalue and n is the number of considered principal components and c is the number of samples.

Fig.4 and Fig.5 show the absorption spectra of a standard sample of Sunset yellow and a spiked saffron sample with Sunset yellow in a series of water-1, 4-Dioxane that solvent components gradual were changed by adding 1, 4-Dioxane into water.

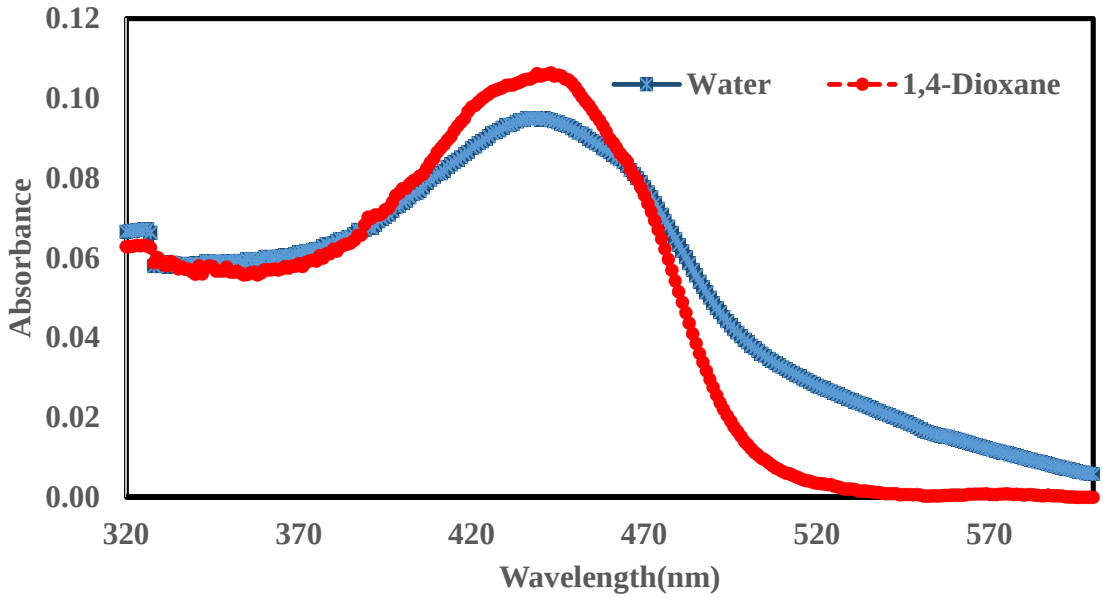


Fig. 2. Normalized Absorption spectra of Saffron in pure water and 1, 4-Dioxane

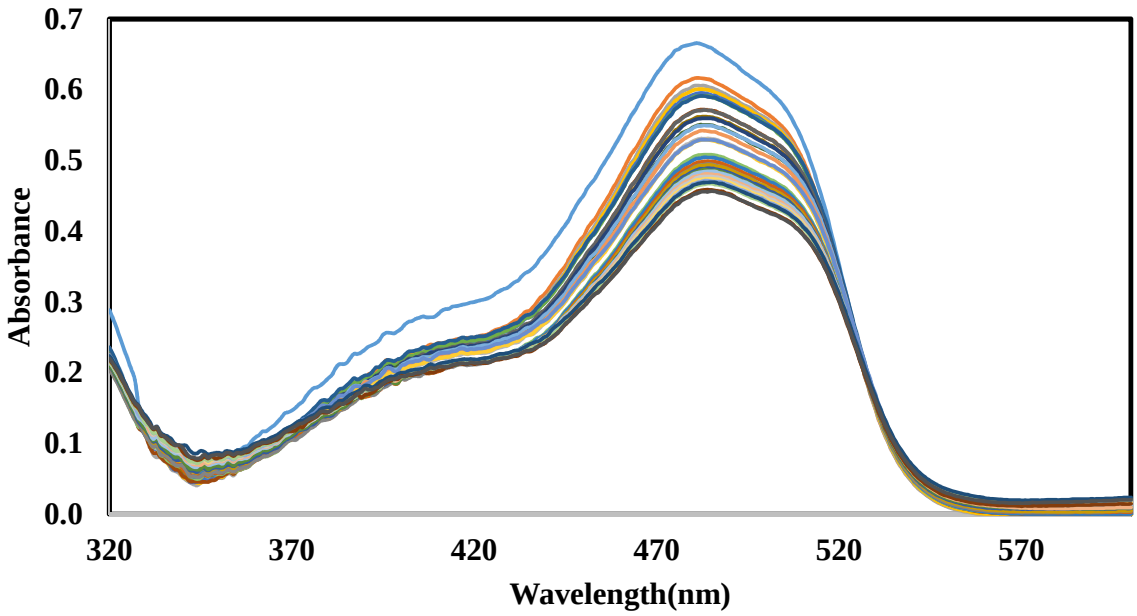


Fig. 4. Absorption spectra of a standard sample of Sunset yellow(13.6 mg mL^{-1}) in a series of water-1, 4-Dioxane mixed solvent. The spectra are recorded after every 25µL of 1, 4-Dioxane added

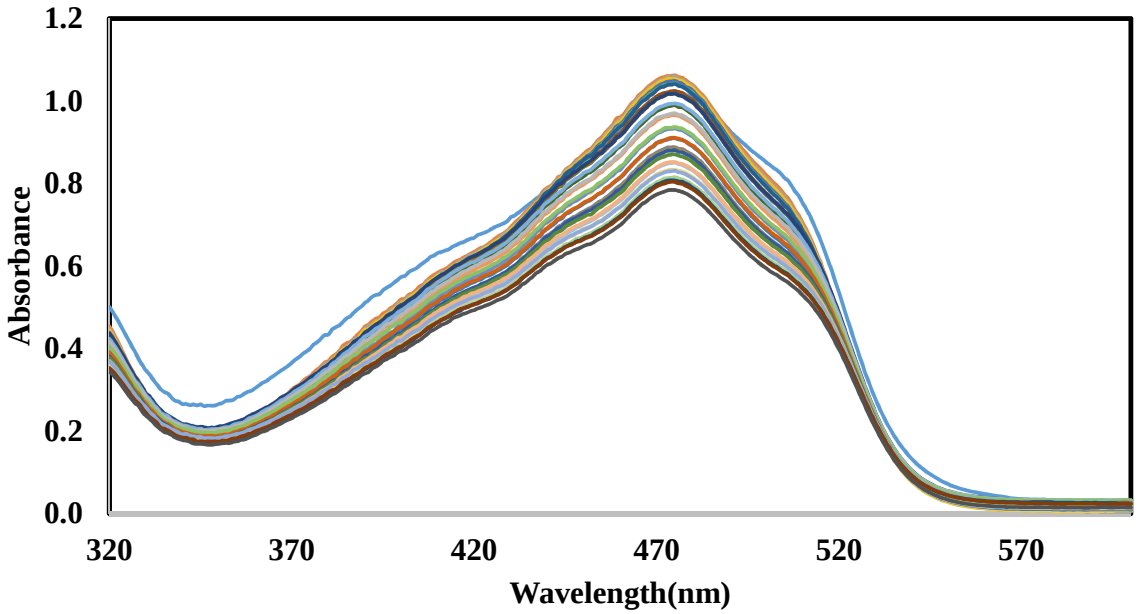


Fig. 5. Absorption spectra of a spiked saffron sample with Sunset yellow (15.8mg mL^{-1}) in a series of water-1, 4-Dioxane mixed solvent. The spectra are recorded after every 25µL of 1, 4-Dioxane added

2.5. Analysis of adulterated Saffron by Sunset yellow using proposed method

Six adulterated Saffron plus one true Saffron samples were analyzed using the proposed method. The amount of Sunset yellow in the spiked samples was selected in the linear range of sunset yellow that was preliminary determined. These samples were used to evaluated applicability and accuracy of the method. The results were summarized in Table 1.

Table1.The results obtained by RAFA on the spiked (adulterated) saffron samples by Sunset yellow

Sample	Actual(mg L^{-1})	Predicted(mg L^{-1})[a]	Recovery (%)
1	Not spiked	Not detected	-
2	10.40	10.21	98.2
3	15.83	16.05	101.4
4	20.09	20.21	100.6
5	18.10	18.19	100.5
6	24.43	24.22	99.1
7	22.62	22.85	101.0

[a]*Average of three measurements.*

From Table 1, it can be seen that the recoveries of Sunset yellow for individual sample are varied between 98.2% and 101.4%. The good agreement between the obtained results and known values indicates the successful applicability of the proposed method in complex samples.

The relative standard errors (RSE) and average recovery values [25,31-32] parameters were selected to assess overall accuracy and prediction ability of the proposed method for determination of sunset yellow in adulterated Saffron.

The average recovery (AR) of Sunset yellow in mixtures can be calculated as

$$AR(\%) = 100 \times \left(\sum_{i=1}^{n} \widehat{c}_i / c_i\right) / n \qquad \text{Eq. (5)}$$

Where n is the number of samples in the prediction set, c_i is the actual concentration of Sunset yellow in the ith sample, and $\widehat{c}_i$ is its estimated value. The prediction error of Sunset yellow in the mixtures can be calculated as the relative standard error (RSEs) of the predicted concentrations:

$$\mathrm{RSE}(\%) = 100 \times \sqrt{\frac{\sum_{i=1}^{n}(\widehat{c_i} - c_i)^2}{\sum_{i=1}^{n}(c_i)^2}} \qquad \text{Eq. (6)}$$

Calculated results of AR and RSE are 100.1% and 1.0%, respectively. These results show that RAFA have good predictive ability for this system.

CONCLUSION

This research investigated a novel procedure for the quantitative detection of Sunset yellow in false saffron. The proposed method is based solvent components gradual change–visible spectra matrices using RAFA as the second order calibration algorithm. In this paper, the complicated operation of pre-separating mixtures (Sunset yellow from crocin, Saffron natural color) is not necessary. The procedure of producing second-order spectra data matrix is simple and fast. The bilinearity of the spectra matrices is dependable and the apparatus used is simple. The proposed method has been successfully used for determination of Sunset yellow in synthetic adulteratedwith satisfactory results.

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