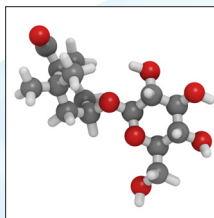


Using Duetta to Detect Saffron Adulteration



Application Note
Life Sciences
FL-2023-07-28



Figure 1: Duetta Fluorescence and Absorbance Spectrometer

Introduction

Saffron is obtained from red stigmas of the *Crocus Sativus* flowers. Thanks to its properties (flavoring, coloring and biological), it has an added value in different industries: Medical, cosmetics, textile, and food. The compounds that are responsible of saffron's color and taste are crocins, which are glycoside derivatives of crocetin, picrocrocin (mainly responsible for the bitter taste), and safranal (monoterpene aldehyde)¹.

Its high price makes saffron a target for counterfeit, especially in its powdered form. Adulteration refers to the addition of extraneous substances (minerals, oils, synthetic or natural colorants from other plants) in the aim of improving its appearance or increasing weight. Different analytical methods like UV-Vis, HPLC, NIRS (Near Infrared Spectroscopy) have been used to detect exogeneous compounds and for ISO certification².



Figure 2: Pistillates of saffron

Duetta is a two-in-one spectrometer that performs both Fluorescence and Absorbance measurements. Thanks to dedicated holders, Duetta enables measuring liquids, as well as powders, reducing preparation time.

Its features³, combined with powerful software make it the ideal tool for saffron analysis and food characterization in general.

Name	Type	Origin
LeSafran	Pistillates	France
Premium	Pistillates	Afghanistan
Frisafran	Powder	Spain
Herbier Alpes	Powder	Unknown
Silk Route	Pistillates	Spain

Table 1: Saffron samples

Five saffron samples from different providers were measured; the above table summarizes their type and origin.

Methods and results

Saffron was prepared in distilled water at 1 mg/mL and incubated 2h30 in the dark. Saffron solutions were then diluted at 1/10 in water before acquisition to avoid detector saturation. EEM spectra were acquired according the following parameters (Figure 3).

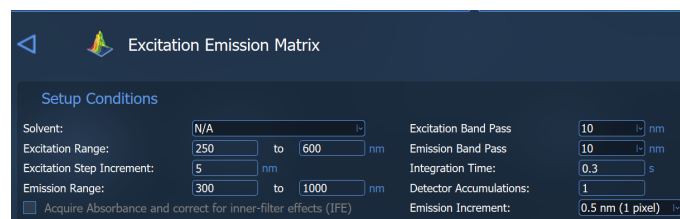


Figure 3: EEMs spectra setup parameters

¹ Masia E., Taitia C., Heimlera D., Vignolinib P., Romanib A., Mancus S. PTR-TOF-MS and HPLC Analysis in the Characterization of Saffron (*Crocus Sativus* L.) From Italy and Iran, *Food Chemistry* 192 (2016) 75–81

² Ordoudi S.A., Cagliani L.R., Melidou D., Tsimidou M.Z. & Consonni R., *Uncovering a Challenging Case of Adulterated Commercial Saffron*, *Food Control* (2017), Doi: 10.1016/J.foodcont.2017.05.046.

³ Application Note: 2 In 1 Fluorescence and Absorbance Spectrometer: How Does It Work?

Results and discussion

The figure below shows the EEMs acquired for each sample.

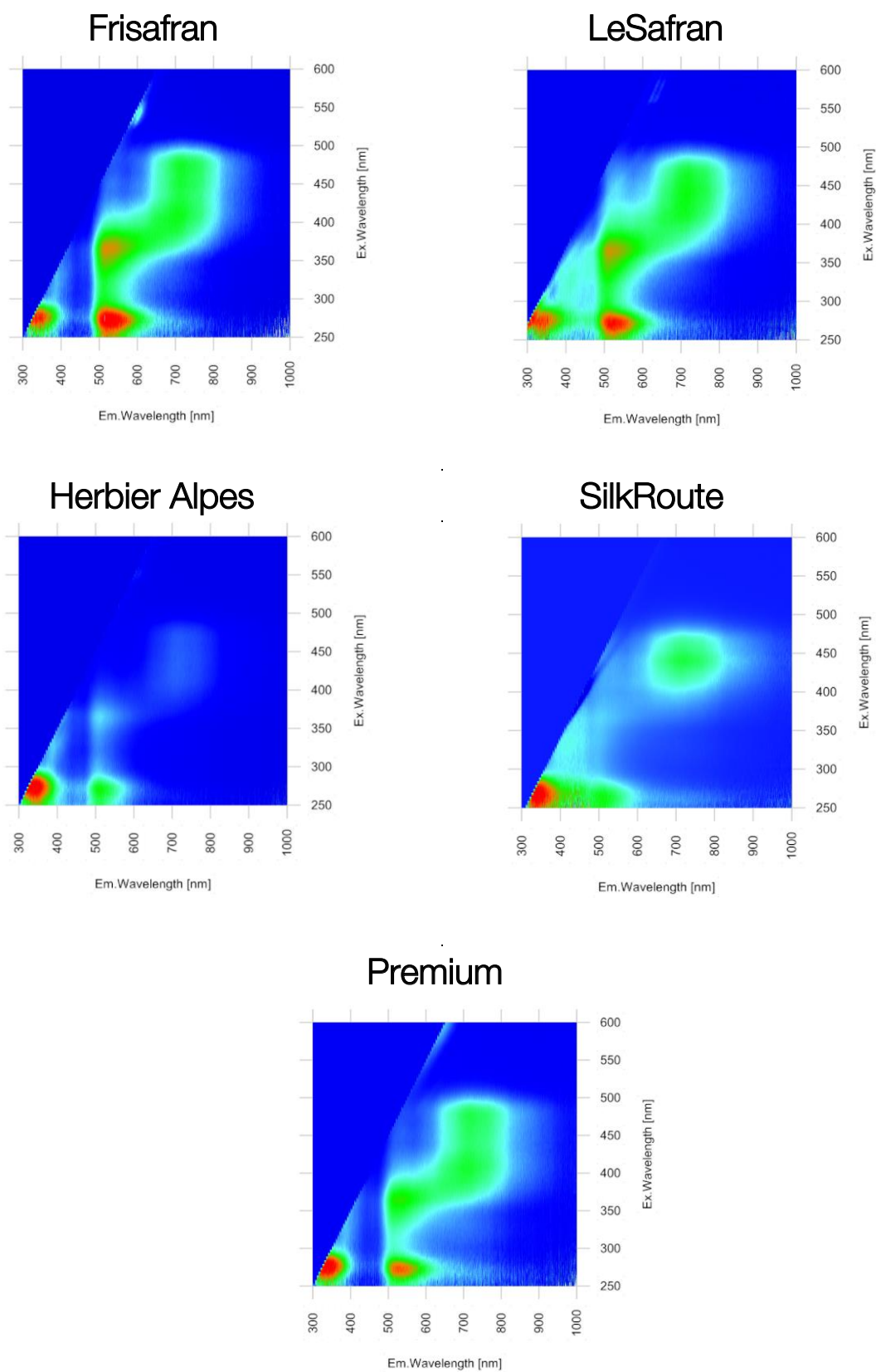


Figure 4: EEMs contour plots for saffron

EEM spectra of saffron point to fluorescence emission activity is in the range of 300-900 nm. Bands between 300 nm and 400 nm, and between 500 nm and 600 nm correspond to safranal and crocin respectively, that are the main components of saffron.

To assess the sensitivity of the instrument to detect adulteration of saffron, curcumin, a commonly used adulterant spice, was chosen to provide a series of mixtures with one of the saffron brands. Mixtures of curcumin and LeSaffron were prepared according to the scheme below in Table 2.

Curcumin	LeSaffron
100%	0%
3%	97%
10%	90%
30%	70%
60%	40%
0%	100%

Table 2. Curcumin-saffron mixture composition

The EEM fingerprints of the series of mixtures of the two compounds, as well as the two compounds themselves, were subjected to PARAFAC analysis (Eigenvector Inc., Washington US). Decomposing into three components accounted for 97.35% of the variance.

The curcumin-saffron mixture EEM series was subjected to a partial least squares regression (PLS) to evaluate the precision of the EEM fingerprint technique in detecting changes in the relative concentrations of the two compounds. The PLS regression with three latent variables (3LV) yields Figure 5 with R² values of Calibration and Cross Validation at 1.000 and 0.996 respectively.

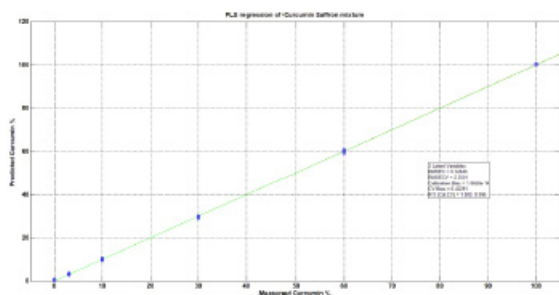


Figure 5: Partial Least Squares (PLS) regression plot of the curcumin-saffron mixture series in Table 2.

The R² parameter values indicate that the EEM technique lends itself to chemometrics analysis to quantitate the level of potential adulteration of saffron with curcumin in the single % range given the EEM fingerprints of the parent compounds of such mixtures.

Splitting the sample series into Calibration and Validation datasets and performing Cross Validation yields the regression plot in Figure 6. With the R² error of Prediction 0.999.

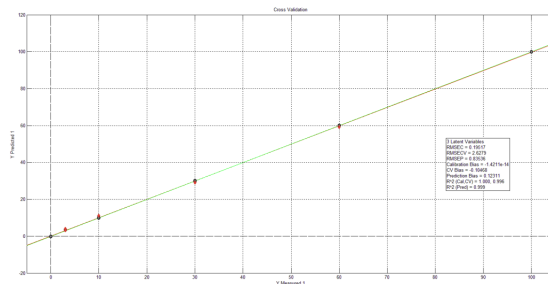


Figure 6: Cross Validation regression plot of the curcumin-saffron mixture series with 3 latent variables. Black dots represent measured calibrating samples, Red dots represent predicted values.

The presented results indicate that the EEM fingerprint technique is able to quantitatively predict the level of adulteration of saffron with curcumin.

Conclusion

Saffron is a target for adulteration due to its high price. Other compounds can be added, such as minerals, oils or synthetic colorants. The two in one Duetta spectrometer was used to rapidly acquire Excitation Emission spectra for different saffron samples from different suppliers. The comparison of emission profiles points out the presence of the main components of saffron: Crocin and Safranal, but shows also some difference that can be due to external components. EEMs can be a tool to differentiate visually, and easily, bad quality samples compared to a reference. In parallel, mixture series were prepared using curcumin, a common adulterant at different percentages to assess the sensitivity of the instrument to detect adulteration of saffron. The results confirm the possibility to quantitatively predict the level of adulteration of saffron with curcumin.

For quantitative work, and for industrial QC/QA implementations of EEM (A-TEEM) spectroscopy, the HORIBA Aqualog A-TEEM spectrometer is highly recommended. Consult with your HORIBA representative for more details.