



Chromatography-free Screening and Quantitation of Saffron Adulteration

Abstract

Direct Analysis in Real Time (DART) is a well-established method for food quality analysis. As a chromatography-free workflow with minimal sample prep, DART allows for high-throughput analysis with a runtime of ≤ 30 sec per sample. The launch of the

EVOQ® DART-TQ+ has prompted investigations for the quantitative analysis of food contaminants and adulterants. Here, a robust, sensitive and rapid method for the quantitative determination of saffron adulteration by safflower is presented.

Keywords:

Chromatography-free; adulteration; DART; EVOQ DART-TQ+; Food

Introduction

Saffron faces rising threat of adulteration

Saffron is a high value spice obtained from drying the stigmas of *Crocus sativus* L. (*Iridaceae* family), mainly produced in India, Iran, and other Mediterranean countries. It is classified as the most expensive spice with prices of \$1,100 -11,000 per kg due to high production and harvesting costs and therefore a prime target for adulteration leading to illicit economic gains. Recently Europol supported Spanish authorities in targeting organized criminal activity for trafficking adulterated saffron. Identified fraud for a value of 10 Million Euro in more than 500 shipments of adulterated saffron was reported¹.

Common additives in saffron adulteration

Aside from creating a profitable illegal business by reducing the quality of saffron, adulteration may also lead to serious health concern. The most common substances illegally added to saffron are powders from turmeric (*Curcuma longa*), safflower (*Carthamus tinctorius*) and paprika as well as natural dyes from flowers and roots, artificial dyes like erythrosine, ponceau 4R, tartrazine, Sudan dyes, magenta III and rhodamine B, or Safranin which is a synthetic aroma compound.

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Limitations of current detection methods

Different methods have been developed for detecting saffron adulteration. Most of them are time consuming and complex since they involve gas chromatography (GC), thin layer chromatography (TLC), liquid chromatography-mass spectroscopy (LC/MS), nuclear magnetic resonance (NMR), or molecular methods (PCR). Untargeted methods coupled with advanced statistical analysis have been also investigated as alternative approach. The official ISO 3632-2 protocol is the current standard method for saffron quality analysis. This method combines UV-vis spectroscopy and LC to determine the color strength and the crocin content. However, this method is unable to detect certain saffron adulterants (safflower, marigold, or turmeric) added to a level below 20% (w/w).

Prevalence of safflower as a saffron adulterant

In Europe, saffron is sold as entire stigmas (Figure 1), considered the best quality product, as powder or as an ingredient in commercially processed foodstuffs (e.g. seasoning mixes). When present as powder

or ingredient, addition of colorants or plant tissues for adulteration can be easily done. Safflower (*Carthamus tinctorius* L.) is an herbaceous, thistle-like plant belonging to the *Asteraceae* family. It is of economic interest since its seeds are an important source of unsaturated fatty acids. The plant has a considerable resistance to salinity when compared to other oilseed crops. Thus, safflower is commonly cultivated in arid and semi arid regions like Kazakhstan, Mexico and India, which produced 52% of the safflower global amount in 2014. These same regions are also the main producers of saffron. Aside of being considered as an emergent oilseed crop, the yellow and red pigments (e.g. carthamin) in the petals of safflower (Figure 2) are commonly used as dyeing agents in the food and textile industry. Because of the deep red color of carthamin, safflower is also frequently used to adulterate saffron.

A rapid DART-based approach for adulteration detection

Here, we developed a rapid, robust, high-throughput DART method for the reliable quantitation of saffron adulteration with low safflower contents on the EVOQ® DART-TQ+.



Figure 1. Saffron stigmas



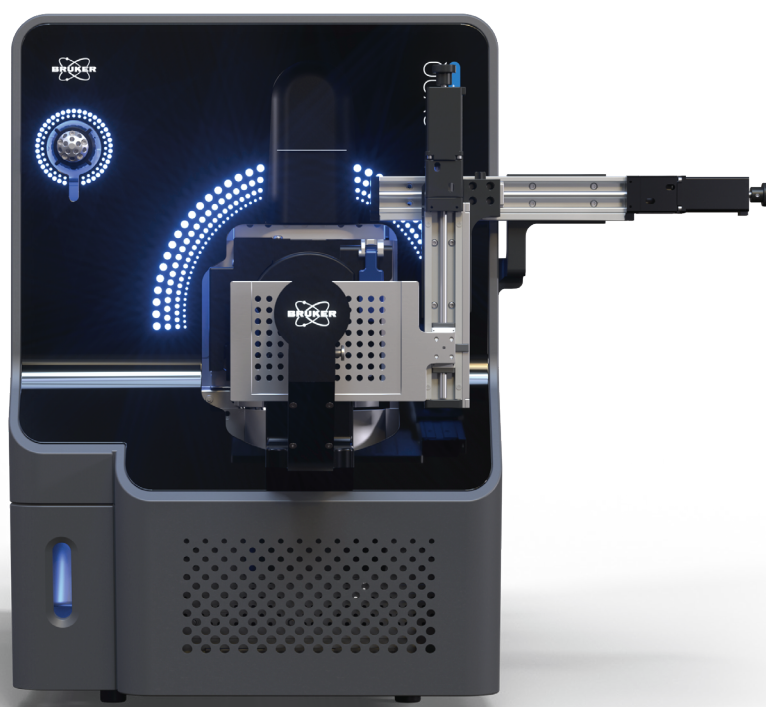
Figure 2. Safflower petals

Methods

Pure saffron was extracted by adding 5 mL of EtOH/H₂O at a 70/30 ratio to 50 mg of powder. It was shaken for 10 min at room temperature, centrifuged for 5 min at 13,000 rpm and filtered on PTFE (0.445 µm). Then a serial addition of 10 - 70% safflower to the saffron extract was performed. An EVOQ® DART-TQ+ (Bruker, Bremen, Germany) was used for the analysis coupled with integrated DART source (Figure 3).

With statistical analyses, potential molecular markers tracing for safflower in saffron were detected in MS full scan mode. For these selected candidates, MRM transitions were optimized. DART parameter settings like temperature, voltages and pressure were investigated as well. The repeatability of the analysis and the influence of an internal standard for the MRM method were examined.

Figure 3. EVOQ® DART-TQ+, equipped with an integrated DART source. Samples are deposited on a QuickStrip® HTS sample card for analysis.



Results

Figure 4 shows typical full scan MS spectra of saffron and safflower. After a careful evaluation of the data, several m/z values were found to be different, i.e. potential discriminant markers at m/z 116.2, 198.2, 296.2 and 446.4. Figure 5 shows a clear correlation of the peak area of both the precursor at m/z 116 and the MRM transition to m/z 70 with increasing addition of safflower. After method optimization, the MRM transitions of 116/70 and 446/116

turned out to be best for the differentiation. An additional High Resolution (HR) MS/MS untargeted analysis allowed to putatively attribute the ion at m/z 116 to proline. For the DART-MS quantitation of these marker ions, the use of caffeine as an internal standard significantly improved the results by compensating the errors and variability of the measurement due to the ionization process, the sample position on the DART grid or MS related factors.

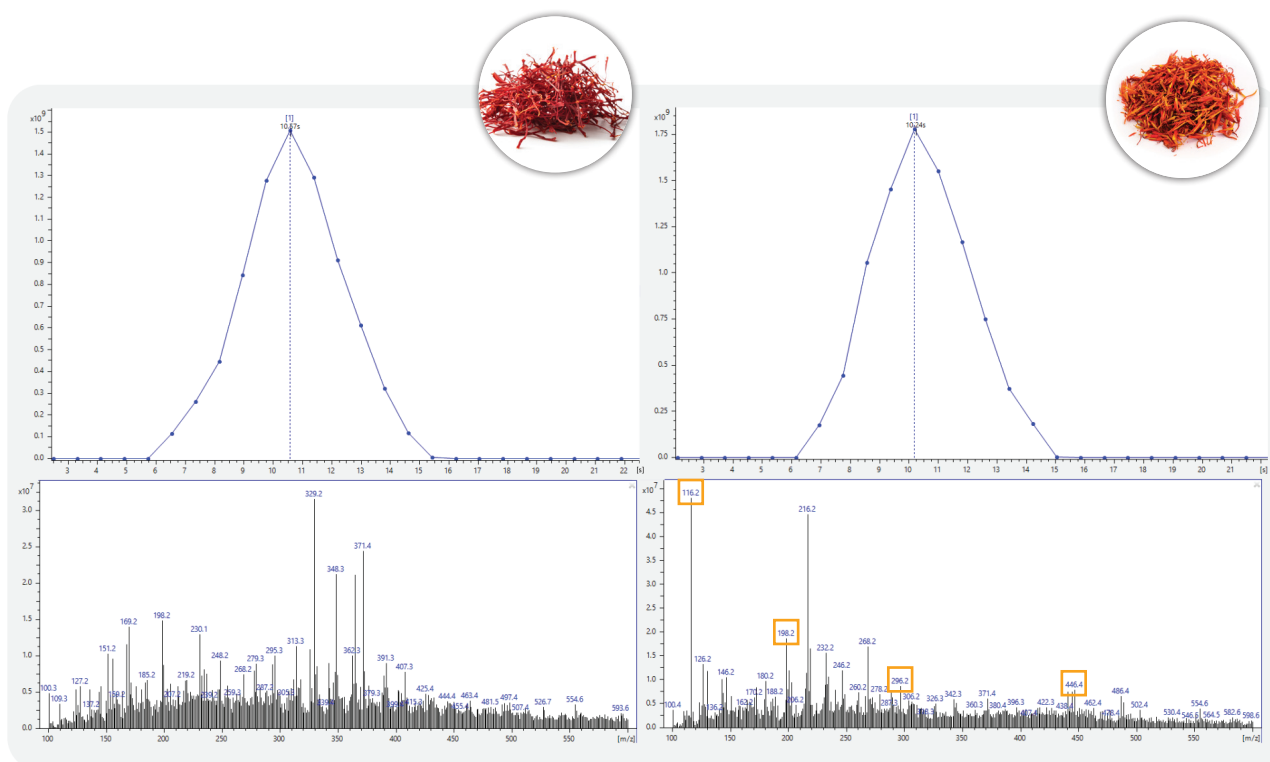


Figure 4. Full scan MS spectra of pure saffron (left) and pure safflower (right). The list of visible discriminant precursor ions includes ions at m/z 116.2, 198.2, 296.2 and 446.4.

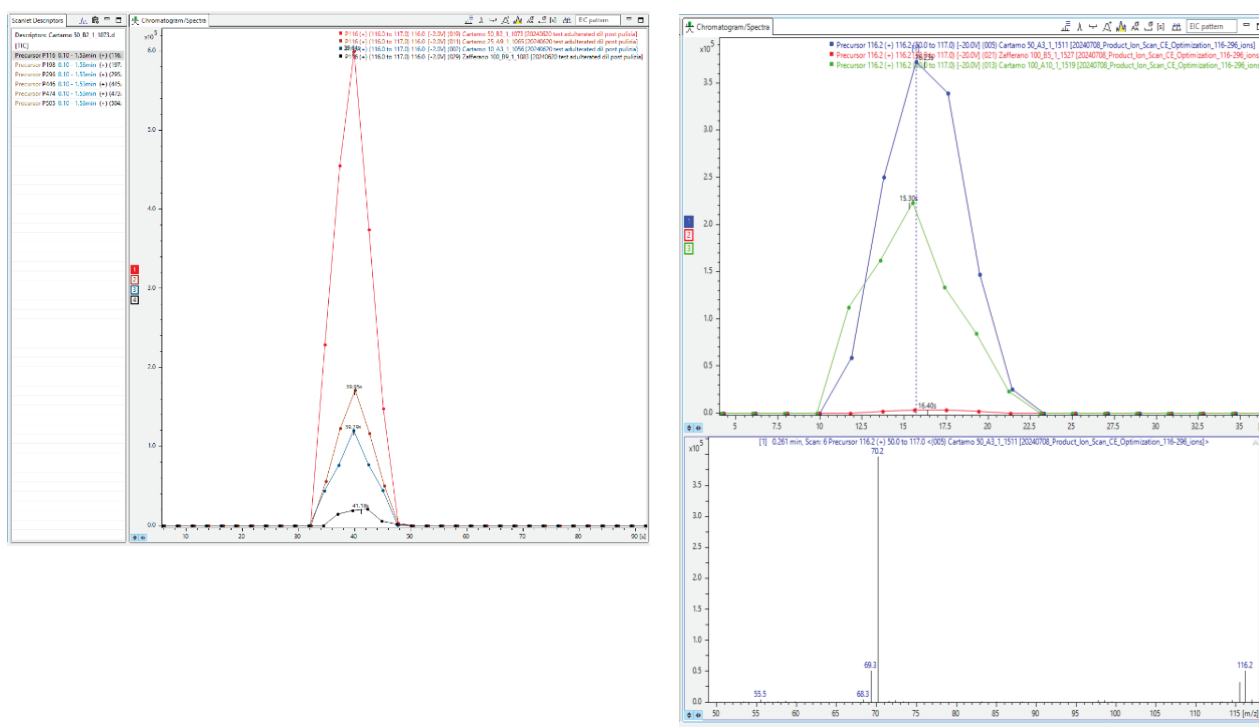


Figure 5. Peak area of both the precursor ion at m/z 116 (top) and the MRM transition m/z 116/70 (bottom) with increasing addition of safflower.

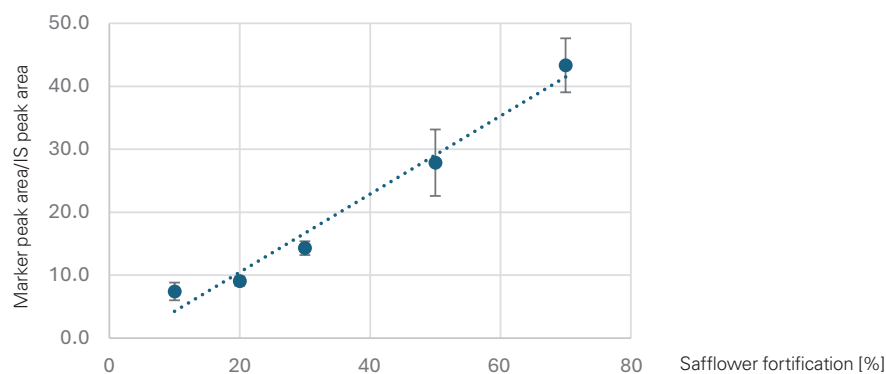


Figure 6. Calibration curve of adding safflower to saffron, using caffeine as internal standard. $R^2 = 0.9755$.

Figure 6 demonstrates the excellent linearity of the calibration curve for the addition of 10-70% safflower to the saffron extract with an $R^2 = 0.9755$. The MRM transitions monitored simultaneously along the data acquisition were m/z 116/70, 446/116 and for caffeine 195/138. For repeatability studies, two levels of 20 and 50% of safflower were spiked into the saffron extract. The calculated CV upon 10 replicated analysis was 7% using caffeine as internal standard. Finally, saffron adulteration with safflower was tested. A simple workflow was applied with a fast extraction of the sample

with MeOH/H₂O and dilution followed by the DART-MRM analysis. For the construction of the calibration curves saffron powder was contaminated with safflower powder at the inclusion levels of 5-10-20 % and analyzed to check detection capability of the targeted DART-MS/MS method. The peak areas of the monitored marker were plotted against the % of adulteration in saffron. Figure 7 shows the data which gave correct assignments of safflower down to a 5% addition with an $R^2 = 0.9955$. The total run time for 30 samples was just 15 min.

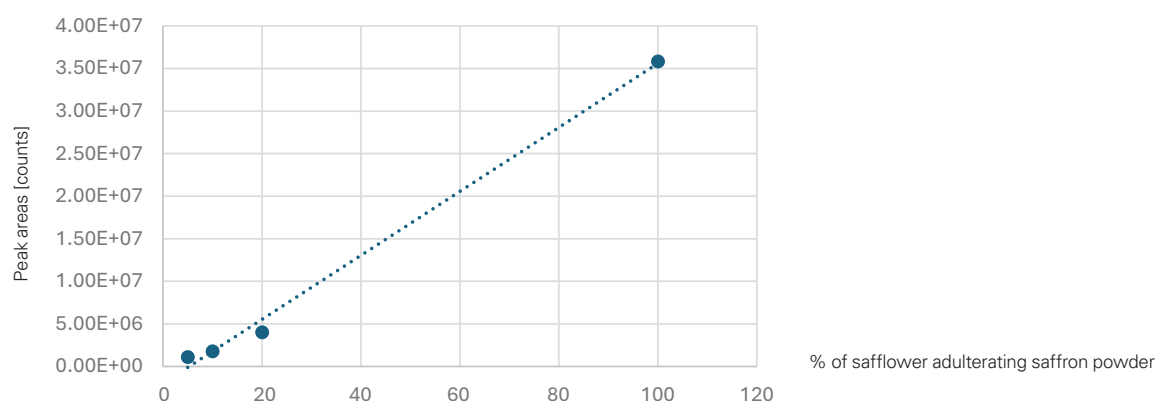
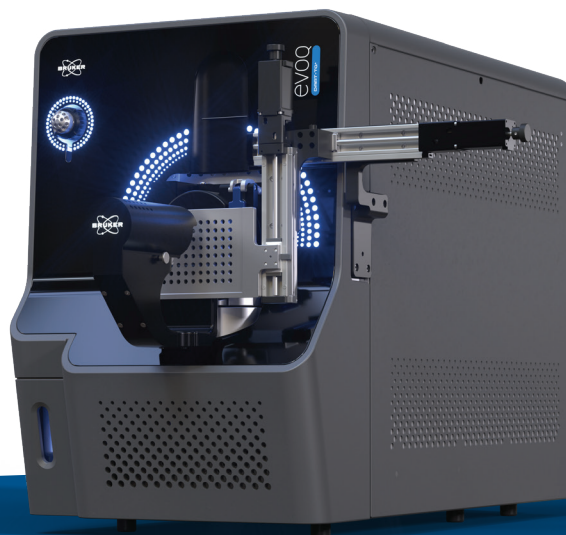


Figure 7. For the construction of the calibration curves, saffron powder was contaminated with safflower powder at the inclusion levels of 5-10-20% and analyzed to check detection capability of the targeted DART-MS/MS method. The peak areas obtained for the monitored marker were plotted against the % of adulteration in saffron and details of the coefficient of the derived regression line were calculated showing a very good correlation coefficient. $R^2 = 0.9955$.



Conclusion

Rapid and reliable quantitation with DART-MS

The optimized method for the chromatography-free DART-MS quantitation of safflower in saffron samples proved to be successful down to the detection at the lowest addition of 5% in real samples. With an easy sample preparation protocol and a run time of 30 seconds per sample, the method is rapid, robust, and reliable. By eliminating chromatographic separation, the DART-MS method enables high throughput, reduces solvent use, and avoids liquid chromatography system maintenance and service. The inclusion of an internal standard significantly contributed to a decreased variability of the measurements and improved the correlation coefficient of the calibration curve.

Future expansion toward multi-target adulterant detection

Further efforts will be directed to extend the method to other potential adulterants in order to deploy a single multi-target method for the detection of several substances used to adulterate saffron.

References

- [1] Europol May 7, 2021 press release: <https://www.europol.europa.eu/media-press/newsroom/news/spice-worth-its-weight-in-gold-multi-million-euros-fake-saffron-trafficking-scheme-uncovered#empact>

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