Application Note

Food and Beverage Testing

Quality Assessment of Saffron by UV-Vis Spectroscopy in Accordance with ISO 3632

Integrated quantitative analysis using an Agilent Cary 3500 UV-Vis and Cary UV Workstation

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Abstract

Saffron is an expensive spice with its price point directly influenced by product quality and authenticity. This makes saffron often subject to adulteration and mislabeling. Commercial saffron can be graded based on its UV-Vis spectrophotometric properties, according to the industry ISO 3632-1:2025 and ISO 3632-2:2010 standards. The results obtained enable classification of saffron into four commercial grades, Extra Class, I, II, and III, providing a standardized approach for quality assurance.

Following the ISO 3632-2 clause 7, 10, and 14 methods, the Agilent Cary 3500 Multicell UV-Vis spectrophotometer was used for the quality grading of a saffron sample by quantifying key bioactive markers. The sample was graded Extra Class, as per ISO 3632-1. The study highlights the benefits of the Agilent Cary UV Workstation software in enabling fast and streamlined saffron quality assessment, and supporting routine quality control workflows, authenticity verification, and consumer confidence.

Introduction

Saffron is the world's most expensive spice, with its price per gram dependent on quality, origin, and purity. It is renowned for its vibrant color and distinctive flavor—attributes that contribute significantly to its culinary appeal. However, saffron's high market value and limited production make it vulnerable to adulteration, quality degradation, and mislabeling, all of which pose a risk to consumer health, safety, and trust.

Ultraviolet-Visible (UV-Vis) spectroscopy is a rapid, reliable analytical technique suitable for assessing spice quality by quantifying bioactive molecular markers. In saffron, crocin is the carotenoid compound responsible for color intensity, picrocrocin is the primary bittering agent, and safranal is the key contributor to aroma.

In this application note, we used an Agilent Cary 3500 UV-Vis spectrophotometer and Agilent Cary UV Workstation software to analyze a commercial saffron sample. The aim of the study was to grade the quality of the sample in accordance with ISO 3632-1:2025 and ISO 3632-2:2010 Spices — Saffron (*Crocus sativus* L.).[1,2] These well-established industry standards provide specifications and test methods for the analysis of dried saffron, including filaments, cut filaments, and powder. The standardized test methods outlined in ISO 3632-2 support the classification and quality evaluation of saffron. The methods relate to the determination of moisture and volatile matter content (clause 7), crushing and sieving of samples for tests (clause 10), and the determination of the main characteristics using a UV-Vis spectrometric method (clause 14). Based on these evaluations and the quality criteria defined in ISO 3632-1:2025, saffron samples can be categorized into four commercial grades: Extra Class (EC), I, II, and III, with EC indicating the highest quality.

Experimental

Sample preparation

Determination of moisture and volatile matter content

First, 2.5 g of saffron filaments (bought locally) were weighed to the nearest 0.1 mg (2.5018 g), in a clean, dry, and tared weighing dish. This test portion was then placed uncovered in an oven and maintained at 100 ±1 °C for 16 hours. After cooling, the sample was reweighed to the nearest 0.1 mg. The moisture and volatile matter content (w_{MV}) was calculated and expressed as a percentage of the initial sample. This value was reported to four decimal places and used in subsequent UV-Vis spectrophotometric analyses.

Determination of the main characteristics of saffron using UV-Vis

Blank: Distilled water (Milli-Q IQ 7005, Sigma-Aldrich, Merck) was used as the blank; 3 mL of distilled water was transferred into a standard 3.5 mL, 10 mm optical pathlength, quartz cuvette (Agilent Technologies, part number 50613387).

Aqueous extract of sample: 1 g of saffron filaments were crushed using a mortar and pestle until 95% of mass fraction could pass through a sieve with a 500 µm pore size mesh. Then, 0.5 g of the crushed sample was weighed to the nearest 0.1 mg, in a clean, dry, and tared weighing dish (0.5330 g), and this was considered the sample test portion weight. This test portion was transferred into a 1,000 mL beaker and 900 mL of distilled water was added. The solution was stirred with a magnetic stirrer for one hour, away from light. After this period, the magnetic bar was removed, and the beaker was made up to the mark with distilled water and mixed. A 20 mL aliquot was taken and transferred into a 200 mL volumetric flask, made up to the mark with distilled water. The flask was stoppered and mixed. The solution was rapidly filtered through a mixed cellulose ester (MCE) filtration membrane of pore size 0.45 µm (HAWP02400, Sigma-Aldrich, Merck) away from light. Finally, 3 mL of the clear solution (aqueous extract of the sample) was transferred into a standard 10 mm quartz cuvette, ready for analysis by UV-Vis.

Instrumentation

The blank cuvette and aqueous extract of sample cuvette were loaded in the Agilent Cary 3500 Multicell UV-Vis (Figure 1), which was operated using the parameters listed in Table 1. The instrument is powered by a xenon flash lamp that requires no warmup time and enables a full spectrum scan to be collected in under a second. The lamp includes a 10-year replacement warranty, minimizing the frequency and cost of replacement. The Cary 3500's modular design approach enables the spectrophotometer series to be configured towards varying sampling flexibility and scalability needs. For single sample applications and laboratories with reduced bench-space, the Cary 3500 Compact UV-Vis allows a user to measure a single sample and reference in ambient or temperature-controlled configuration. This application can also be conducted on the Cary 3500 Flexible UV-Vis, which has a larger-sized compartment for liquid and solid sample measurements, and for use with accessories.

After establishing the baseline by measuring the blank, the UV-Vis profile of the aqueous saffron extract was collected.

Figure 1. The Agilent Cary 3500 Multicell UV-Vis spectrophotometer has eight stationary cell positions and a double beam optical design with fiber optic technology and eight respective detectors, allowing simultaneous measurement of up to seven samples and a reference.

Table 1. UV-Vis data collection parameters for saffron analysis using an Agilent Cary 3500 Multicell UV-Vis spectrophotometer.

Setting	Parameter
Wavelength Range	200–700 nm
Analysis Wavelengths	257, 330, 440 nm
Signal Averaging Time	0.020 sec
Data Interval	1 nm
Spectral Bandwidth	2 nm

Results and discussion

Determination of moisture and volatile matter content

The moisture and volatile content (w_{MV}) of the sample was calculated as 6.43% of the initial weight. According to ISO 3632-1:2025, this value classifies the saffron sample as EC, as shown in Table 2. The w_{MV} value is also used in the determination of saffron's main characteristics, as described in the next section.

Table 2. Calculations of moisture and volatile content.

Characteristic	Sample		Unit	
Test Portion (m0)	2.5018		g	
Dry Residue (m4)	2.3410		g	
Moisture and Volatile Matter Content (w_{MV})	6.4274		%	
Grading				
Specification Categories	**EC**	**I**	**II**	**III**
In Filament Form	<12%			
In Powder Form	<10%			
Sample Specification Categorization	Extra class			

Determination of saffron main characteristics using a UV-Vis spectrometric method

The UV-Vis spectrum of saffron was collected across the 200 to 700 nm wavelength range using the Cary 3500 Multicell UV-Vis, as shown in Figure 2. The results of the three primary saffron constituents were obtained by direct reading of the specific absorbance at the following three wavelengths:

- $A^{1\%}_{1cm}$ (257 nm): absorbance at ~ 257 nm (λ_{max} of picrocrocin)
- $A^{1\%}_{1cm}$ (330 nm): absorbance at ~ 330 nm (λ_{max} of safranal)
- $A^{1\%}_{1cm}$ (440 nm): absorbance at ~ 440 nm (λ_{max} of crocin)

Quantitative grading of the saffron sample was performed in accordance with ISO 3632-1:2025, which defines quality parameters based on the absorbance strength of the three constituents. Once the absorbance values had been recorded at these wavelengths to four decimal places, the strength values were calculated using methodology described in ISO 3632-2:2010.

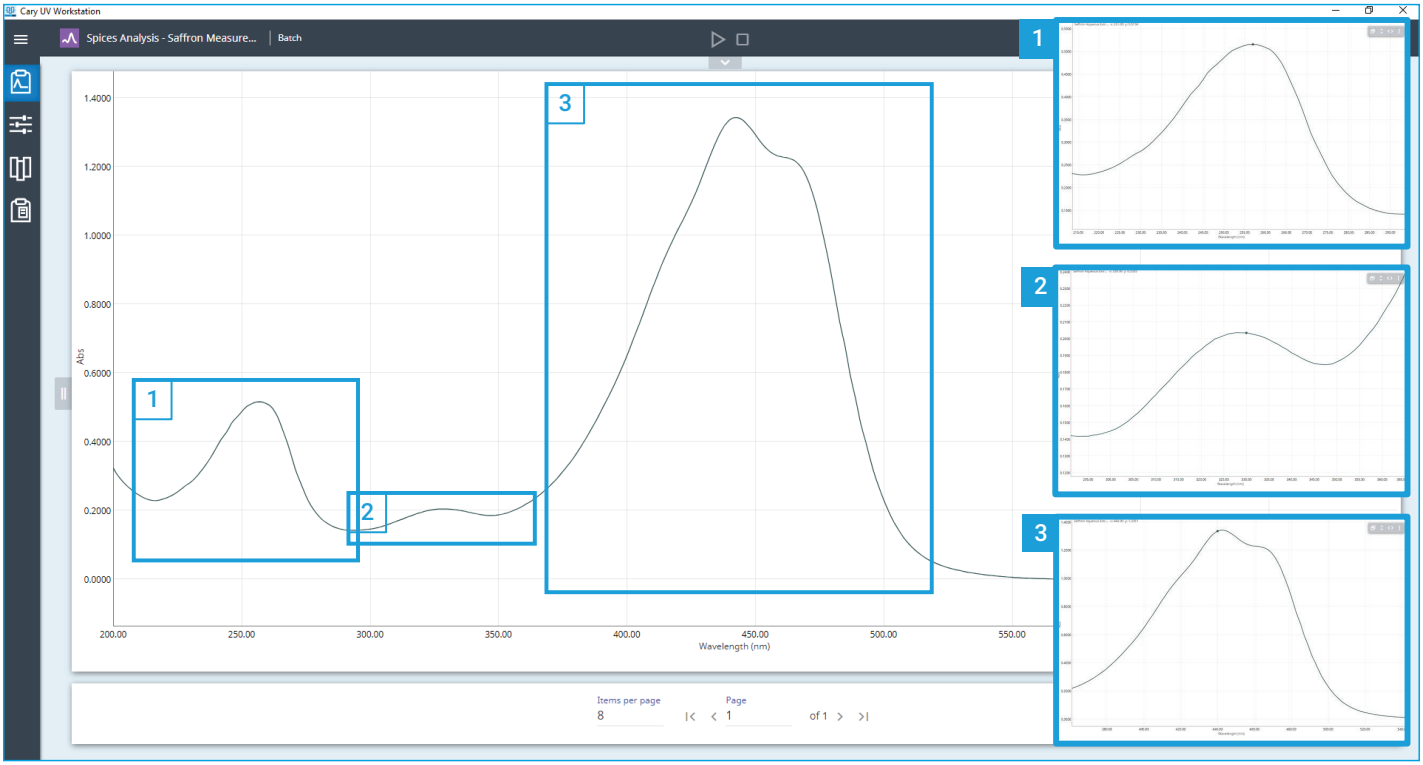

Figure 2. UV-Vis spectrum ranging from 200 to 700 nm of aqueous extract of saffron. (A) shows absorbance maximum of picrocrocin, (B) shows the characteristic shoulder shape of safranal around 330 nm, and (C) shows the characteristic spectrum shape of crocin absorbance.

The strength of a saffron constituent can be calculated using Equation 1.

Equation 1.

$$A\frac{1\%}{1cm}(\lambda_{max}) = \frac{D \times 10\,000}{m \times (100 - w_{MV})}$$

where:

D = the specific absorbance of the constituent

10 000 = the unit conversion factor allowing comparison across different saffron samples

m = the mass, in grams, of the test portion

w_{MV}= the moisture and volatile matter content, expressed as a percentage mass fraction, of the sample

The constituents' calculations were expressed using the following equations:

Equation 2.

$$(\text{picrocrocin, taste agent}) = \frac{D(257\text{ nm}) \times 10\,000}{0.5330 \times (100 - 6.4274)}$$

Equation 3.

$$(\text{safranal, aroma agent}) = \frac{D(330\text{ nm}) \times 10\,000}{0.5330 \times (100 - 6.4274)}$$

Equation 4.

$$(\text{crocin, coloring agent}) = \frac{D(440\text{ nm}) \times 10\,000}{0.5330 \times (100 - 6.4274)}$$

These calculations provide values for the classification of taste, aroma, and coloring strength of the saffron sample, enabling it to be graded as EC, I, II, or III per ISO 3632-1:2025 specifications.

The analysis of the data was performed using the Cary UV Workstation software, which enabled both data acquisition and automated calculation of strength values within the same platform.

As shown in Figure 3, the sequence setup page allowed the integration of custom parameters, such as sample mass, and the implementation of the following three calculations using the "End of sequence" analysis tool:

- Analysis 1: Taste strength (picrocrocin)
- Analysis 2: Aroma strength (safranal)
- Analysis 3: Coloring strength (crocin)

Upon completion of the scan, the Cary UV Workstation generated a scalable spectrum and analysis tables displaying absorbance and calculated strength values for each compound. The absorbance values obtained for picrocrocin, safranal, and crocin compounds in the test sample (0.5330 g) are shown in Table 3.

Table 3. Absorbance results of picrocrocin, safranal, and crocin compounds within the aqueous extract of saffron (0.5330 g of sample).

Analyte	λ	Abs
Picrocrocin	257	0.5156
Safranal	330	0.2035
Crocin	440	1.3351

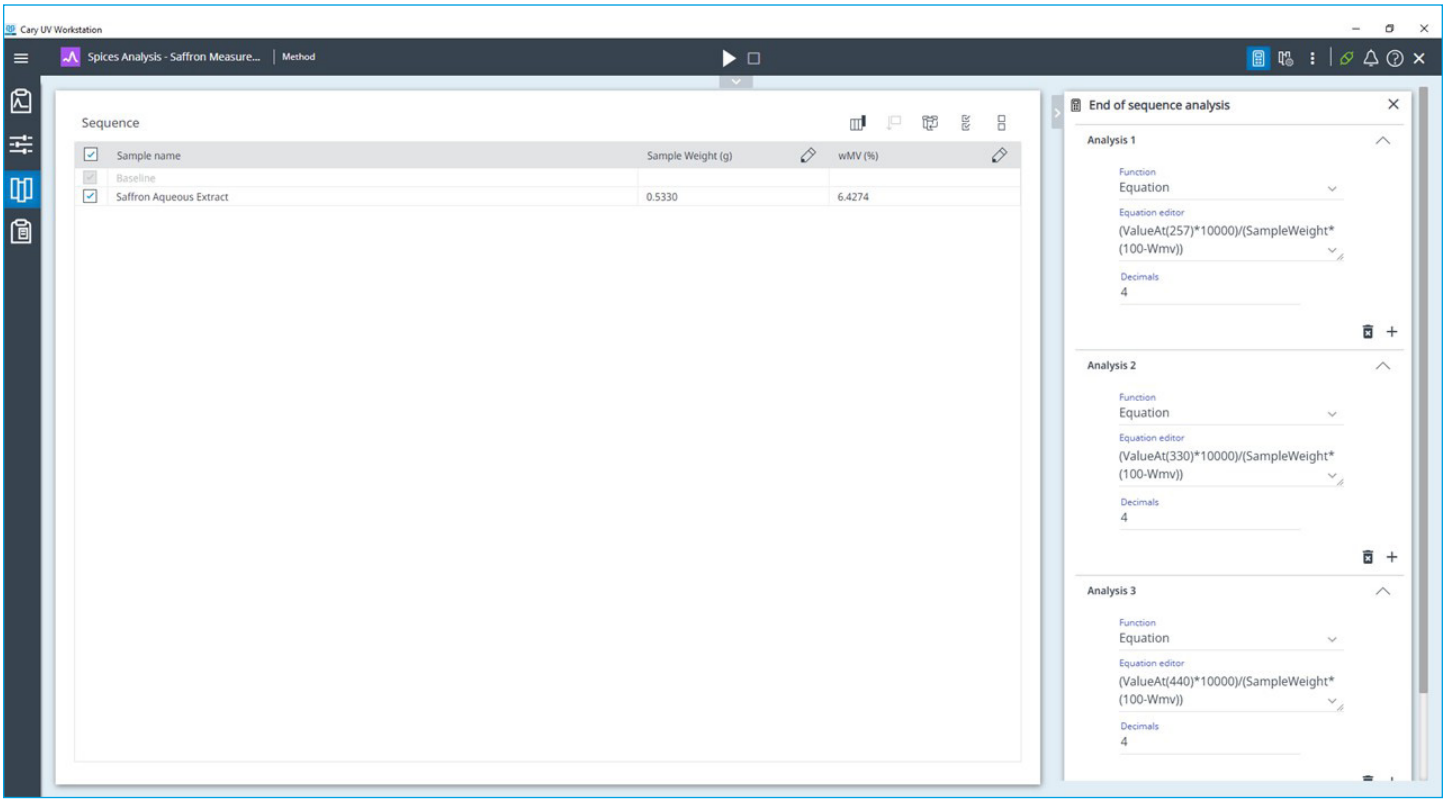

Figure 3. The sequence setup page in the Agilent Cary UV Workstation, which allows a user to create the test and calculation methods. Sample weight was added as a custom parameter and the three equations were input into the End of sequence analysis function, enabling the automatic calculation of the main characteristics of saffron.

These absorbance values were processed to determine the classification of the saffron sample based on ISO-defined minimum (and maximum) strength requirements. The absorbance values (Figure 4, Wavelength scan) and an example of the calculation of taste strength, based on the absorbance at 257 nm for picrocrocin (Figure 4, Analysis 1) is shown.

The Cary UV Workstation transformed the UV-Vis spectroscopy data into commercially useful information. Using automated quantitative analysis, which can be saved as an accessible method, the software provided a numeric grading of the sample according to the ISO 3632-defined commercial classification system.

As shown in Table 4, all three characteristics of the saffron extract met the EC requirements according to ISO 3632-1:2025, confirming the sample's high quality. The moisture and volatile matter (w_{MV}) content, determined separately at 6.43%, was also within acceptable limits for EC classification. This value was also included in the strength calculations.

Overall, the Cary UV Workstation provided a streamlined, user-friendly workflow by integrating data collection, spectral analysis, and ISO-compliant calculations into a single platform. This automated approach reduces manual errors and simplifies saffron grading for research and commercial quality control applications.

Figure 4. Results of Analysis 1, taste strength of the saffron aqueous extract, and specific absorbance of the three primary saffron constituents, automatically calculated using the Agilent Cary UV Workstation software.

Table 4. Strengths and grading results of picrocrocin, safranal, and crocin compounds within the aqueous extract of saffron.

Characteristic	λ (nm)	ISO Strength Specifications	EC	I	II	III	Calculated Strength	Sample Grading
Taste Strength	257	Minimum	80	70	55	40	103.3773	Extra class
Aroma Strength	330	Minimum	30	20	20	20	40.8105	Extra class
		Maximum	50	50	50	50		
Coloring Strength	440	Minimum	230	200	170	120	267.6997	Extra class

Reporting

Accurate, traceable documentation is essential for analytical reproducibility, quality assurance, and compliance. The Cary UV Workstation facilitates this traceability through an integrated reporting system. These reports can be generated in PDF and/or CSV format, minimizing transcription errors.

A CSV file format was first exported (Figure 5) to a custom location, detailing method, sequence and sample parameters, and calculation results. The CSV export facilitates laboratory data transfer into LIMS, facilitating quick sample tracking, workflow management, and data accuracy.

The software also auto-generates intuitive Scan reports, which consolidate critical information such as instrumentation parameters, method setup, sample information, absorbance spectra for target analytes, and quantitative results (Figure 6).

Analysis 1 results	2025-08-18 22:44:16 (-07:00)			
(Value at(257)*10000)/(SAMPLEWEIGHT*(100-WMV))				
Sample Name	Results			
Saffron Aqueous Extract	103.377			
Analysis 2 results	2025-08-18 22:44:16 (-07:00)			
(Value at(330)*10000)/(SAMPLEWEIGHT*(100-WMV))				
Sample Name	Results			
Saffron Aqueous Extract	40.8105			
Analysis 3 results	2025-08-18 22:44:16 (-07:00)			
(Value at(440)*10000)/(SAMPLEWEIGHT*(100-WMV))				
Sample Name	Results			
Saffron Aqueous Extract	267.7			
Wavelength scan	2025-08-18 22:44:16 (-07:00)			
Sample	257.00 nm	330.00 nm	440.00 nm	Y mode
	Value	Value	Value	
Saffron Aqueous Extract	0.51559	0.20354	1.33513	Abs

Figure 5. The Agilent Cary UV Workstation generates a CSV-format export detailing End of sequence analyses and results, supporting traceability requirements and streamlining data management.

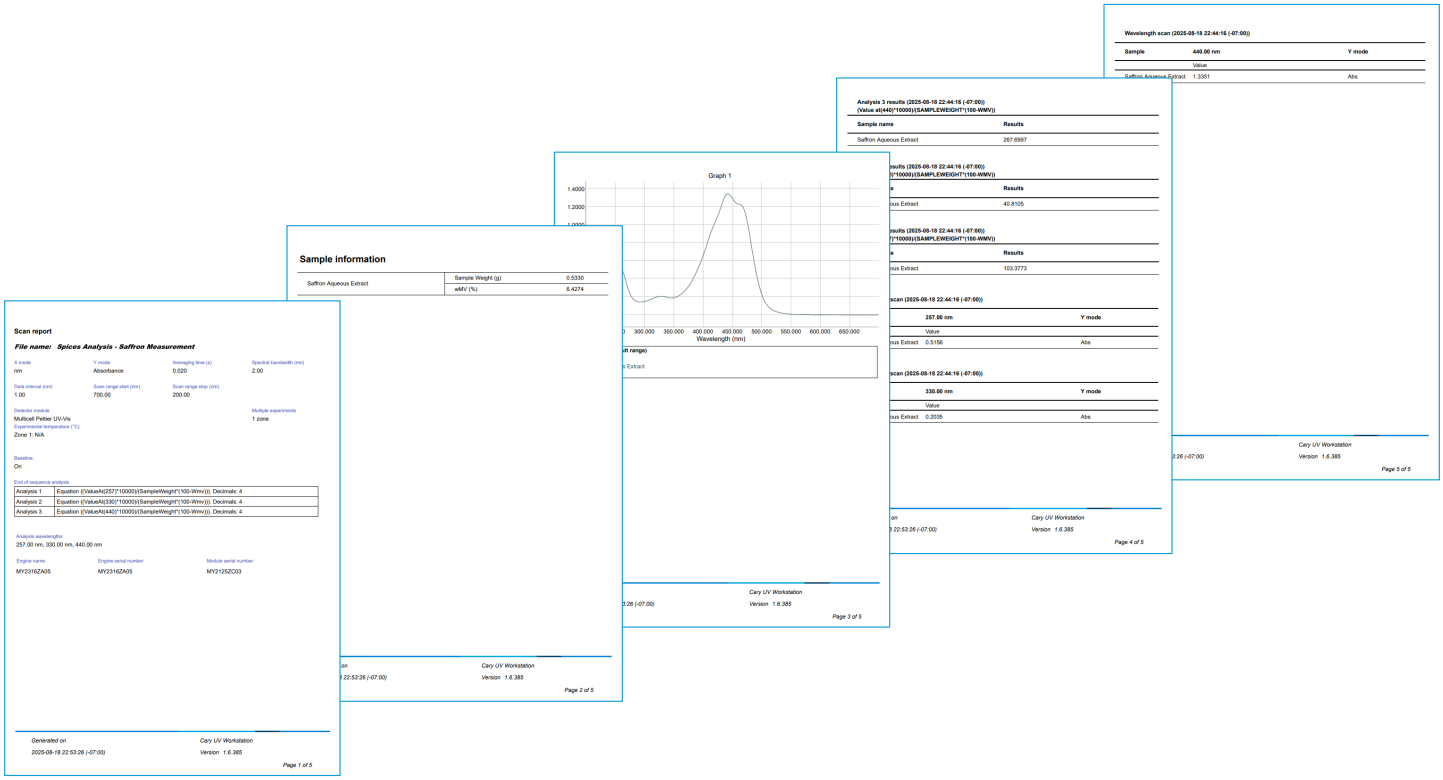

Figure 6. Example of a detailed Scan report generated by the Agilent Cary UV Workstation for saffron measurement.

Conclusion

The Agilent Cary 3500 Multicell UV-Vis spectrophotometer combined with Agilent Cary UV Workstation software provided precise quantification of color, taste, and aroma through the measurement of crocin, picrocrocin, and safranal compounds, respectively. The method supports the quality assessment of saffron in accordance with the ISO 3632 industry standard, enabling reliable grading of commercial samples while also flagging potential cases of adulteration, degradation, or mislabeling.

The Cary 3500 UV-Vis uses a reliable, long-lasting xenon flash lamp that requires no warmup time, allowing immediate operation and acquisition of full spectral scans for blank and saffron samples in under a second. These capabilities support high-throughput analysis of spices through the precise measurement of compound-specific absorbance peaks.

Using built-in spectral tools, automated calculations, and clear reporting features, the Cary UV Workstation significantly simplified the sampling-to-reporting workflow for saffron analysis. Customized calculations defined in ISO 3632 accelerate data processing and reduce calculation errors. The software also delivers results via a user-friendly interface, ideal for traceability.

Together, the Cary 3500 UV-Vis and Cary UV Workstation delivered significant time-savings by minimizing time spent analyzing data and allowing more time for quality assurance of samples. The Cary 3500 system provides an efficient solution for the determination of the main characteristics saffron in accordance with clause 14 of ISO 3632-2:2010.

References

1. International Organization for Standardization, ISO 3632-1:2025 – Spices — Saffron (Crocus sativus L.), Part 1: Specification, ISO, Geneva, Switzerland, 2025
2. International Organization for Standardization, ISO 3632-2:2010 – Spices — Saffron (Crocus sativus L.), Part 2: Test methods, ISO, Geneva, Switzerland, 2010

Further information

- Cary 3500 Multicell UV-Vis Spectrophotometer
- Cary UV Workstation software
- UV-Vis Spectroscopy & Spectrophotometry FAQs

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