AOAC SMPR® 2021.014

Standard Method Performance Requirements (SMPRs®) for Targeted Testing (TT) of Saffron Adulterants

Intended Use: Surveillance and Monitoring by Trained Analysts

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, single-laboratory validation (SLV), or multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholders composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC method review experts, including expert review panels (ERPs), in their evaluation of validation study data for methods being considered for AOAC *Performance Tested Methods℠, Reviewed and Recognized℠,* or AOAC *Official Methods of Analysis℠*, and can be used as acceptance criteria for verification at user laboratories (1).

2 Applicability

The document contains assessment parameters on the performance of targeted testing (TT) methods to monitor saffron powder (as a spice) for the presence of the following potential economically motivated adulterants (EMAs): beet, pomegranate fibers, red dyed silk fibers, safflower and marigold to red stigma of saffron, dyed corn stigmas, gardenia, meat fibers, gelatin fibers, curcuma, sandalwood, Campeche wood powder, stigmas of other saffron types, flowers, starch and glucose, acid orange II, metanil yellow, Sudan I, Ponceau 4R, and Ponceau 6R.

3 Analytical Technique

TT method(s) to monitor saffron powder for the presence of the following potential EMAs: beet, pomegranate fibers, red dyed silk fibers, safflower and marigold to red stigma of saffron, dyed corn stigmas, gardenia, meat fibers, gelatin fibers, curcuma, sandalwood, Campeche wood powder, stigmas of other saffron types, flowers, starch and glucose, acid orange II, metanil yellow, Sudan I, Ponceau 4R, and Ponceau 6R.

TT method to be used to monitor and enforce regulatory requirements for saffron adulterants in food.

Any quantitative method capable of detecting, identifying, and quantifying the presence of an adulterating ingredient in saffron powder present in the food item will be considered.

The scope of the TT method will be defined by the authentic samples and or reference standard material (if available) that were used in validating the method.

4 Definitions

Applicability statement.—The document contains assessment parameters on the performance of TT methods to be used to monitor saffron powder (spice) for the presence of the following potential EMAs: beet, pomegranate fibers, red dyed silk fibers, safflower and marigold to red stigma of saffron, dyed corn stigmas, gardenia, meat fibers, gelatin fibers, curcuma, sandalwood, Campeche wood powder, stigmas of other saffron types, flowers, starch and glucose, acid orange II, metanil yellow, Sudan I, Ponceau 4R, and Ponceau 6R.

Authentic saffron.—Saffron quality is established by ISO based on aroma, bitterness, and coloring strength. ISO 3632-1:2011 (2) establishes saffron quality as one that uses UV-Vis spectrophotometric method that quantifies flavor strength (expressed as concentration of picrocrocin), aroma strength (concentration of safranal), and coloring strength (concentration of crocin).

Economically motivated adulteration (EMA).—Fraudulent addition of nonauthentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller.

Multilaboratory validation (MLV).—Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories according to guidelines in OMA *Appendix D* (3) to be considered for classification as AOAC Final Action Method and in refs. 4–7.

The predicted relative standard deviation of reproducibility ($PRSD_R$) is calculated from the Horwitz equation:

$$PRSD_R = 2C^{-0.15}$$

where C is expressed as a mass fraction.

For quantitative methods undergoing MLV, 10–2 laboratories must be recruited to provide at least eight valid data sets; two blind duplicate replicates at five concentration levels for each analyte/matrix combination to each collaborator.

$$\text{HorRat (repeatability, r)} = RSD_r/PRSD_R$$

$$\text{HorRat (reproducibility, R)} = RSD_R/PRSD_R$$

For interlaboratory studies, acceptable HorRat (R) of 1 with limits of acceptability of 0.5–2; for within-laboratory studies, acceptable HorRat (r) of 0.3–1.3

Nonauthentic substance or adulterant.—Food item intentionally labeled as saffron when the product developer knows that another substance or an adulterant, such as those listed in the applicability statement, has been used to adulterate saffron for economic gain, e.g., saffron of unknown origin labeled as being cultivated in the Protected Designations of Origin (PDO) region in Spain can be used for substitution.

It should be noted that the maximum limit of permissible colors that may be added to any food to be consumed as specified in the Prevention of Food Adulteration Act of India (PFA) is 100 mg/kg body weight.

Metanil yellow, the most frequently and widely used nonpermitted food color that includes synthetic dyes, such as auramine, lead chromate, rhodamine, sudan-3, sudan-4, orange 2, and malachite green, is suspected to be mutagenic and carcinogenic and, therefore, presents potentially serious health issues to the consumer.

Saffron.—Dried stigmas of *Crocus sativas* L. It is cultivated in some regions of Asia (Kashmir, northern Iran) and Europe (Castilla la Mancha, Spain; Kozani, Greece; Abbruzo and Sardinia, Italy). It is one of the most precious agricultural products and most expensive spice amongst 85 known spices in the world. It is a sterile triploid plant, a member of the *Iridaceae* family called red gold.

Table 1. Method performance requirements for color and noncolor adulterants in saffron

Parameter	Acceptance criteria for color adulterants	Acceptance criteria for noncolor adulterants
Analytical range, %	1–30	1–30
LOQ, %	≤1	≤1
Recovery, %	80–120	80–120
Accuracy, %	±20	±20
Precision (repeatability) RSD_r	15	15
Precision (reproducibility) RSD_R	20	20
	Beet, pomegranate fibers, red dyed silk fibers, safflower and marigold to red stigmas of saffron, dyed corn stigmas, stigmas of other saffron types, *curcuma*, acid orange II, metanil yellow, sudan I, Ponceau 4R, and 6R	Gardenia, meat fibers, gelatin fibers, sandalwood, Campeche wood powder, starch and glucose used as fillers or bulking agents

Each saffron flower has only three stigmas, which is used as a food additive due to its aroma, color, and bitter taste. It is traditionally cultivated and harvested by hand, a very time consuming and laborious process. For example, it requires harvesting 150,000 flowers to generate 1 kg saffron.

The quality of saffron depends on the color produced by the carotenoid derivatives crocin and crocetins. The main volatile component of safranal is a monoterpene with molecular formula $C_{10}H_{14}O$, and the bitter taste is produced by the monoterpene glucoside with molecular formula $C_{16}H_{26}O_7$.

Several PDOs have been created to protect the authenticity of saffron as it has, for example, in the Italian region of "Zafferano dell Aquila," one of the major areas of production and global exports.

Single-laboratory validation (SLV).—Demonstration by one laboratory of method performance on samples described according to internationally accepted validation guidelines contained in guidance documents (3, 8–14).

Once the method has been demonstrated to meet the minimum requirements for validation and fit for purpose criteria, the method can be reviewed and considered by AOAC INTERNATIONAL for adoption and publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* as First Action status.

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks and appropriate check standards.

7 Reference Materials

A detailed description of the process used to obtain and evaluate authentic/reference standard materials (sources) and of the test protocol used for validating the method must be provided.

8 Validation Guidance

(a) Data demonstrating method performance is required.

(b) *Samples.*—Complete documentation for the collection and use of authentic samples must be supplied by the method authors. The scope of "authentic" samples used to validate the method must be applicable to the defined scope of the TT method. Expansion of the scope is possible with the inclusion of additional authentic samples and abbreviated validation using the protocol listed in the SMPR.

(c) For SLV studies, the method will be evaluated using prescribed adulterated materials as shown in Table 1. Methods approved at this level will proceed to a second level of evaluation (MLV), where blinded samples containing unknown adulterants will be sent to participating laboratories.

(d) Statistical analysis of interlaboratory studies. Sample size needed to meet performance requirement on proportion.

9 Maximum Time-to-Results

None.

10 References

(1) "Appendix F: Guidelines for *Standard Method Performance Requirements*" (2019) *Official Methods of Analysis of AOAC INTERNATIONAL,* 21st. Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_f.pdf

(2) ISO 3632-1:2011 (2011) *Spices—Saffron (Crocus sativus L.)—Part 1: Specification*, International Organization for Standardization, Geneva, Switzerland

(3) "Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis" (2019) *Official Methods of Analysis of AOAC INTERNATIONAL,* 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_d.pdf

(4) Horwitz, W. (1995) *Pure Appl. Chem*. **67**, 331–343

(5) Codex Alimentarius Committee Guidance Document CAC/GL 27-1997 (1997) *Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food,* http://www.fao.org/input/download/standards/355/CXG_027e.pdf

(6) CAC/GL 37-2001 (2001) *Harmonized IUPAC Guidelines for the Use of Recovery Information in Analytical Measurement,* http://www.fao.org/input/download/standards/376/CXG_037e.pdf

(7) Thompson, M., Ellison, S., Fajgelj, A., Willetts, P., & Wood, R. (1999) *Pure Appl. Chem*. **71,** 337–348

(8) ISO/IEC 17025:2017 (2017) *General Requirements for the Competence of Testing and Calibration Laboratories,* International Organization for Standardization, Geneva, Switzerland, https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:en

(9) CAC/GL 71-2009 (2009) *Guidelines for the Design and Implementation of National Regulatory Food Safety*

Assurance Program Associated with the Use of Veterinary Drugs in Food Producing Animals (Revision 2012, 2014) http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

(10) CAC/GL-49-2003 (2003) *Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis,* http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

(11) Thompson, M., Ellison, S.L.R., & Wood, R. (2002) *Pure Appl. Chem*. **74**, 835–855, http://publications.iupac.org/pac/2002/pdf/7405x0835.pdf

(12) CAC/GL 56-2005–CXG 56 (2005) *Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation, and Quantitative Analysis of Residues,* http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

(13) Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P., & Stein, S. (2003) *J. Am. Soc. Mass Spectrom.* **14**, 528–541

(14) SANTE/12682/2019 (2019) *Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed,* https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf

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