AOAC SMPR 2021.XXX; Draft AOAC Standard Method Performance Requirements (SMPRs) for Targeted Testing (TT) of Saffron Adulterants; Version 2; May 27, 2021

Intended Use

AOACI *SMPRs*® describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be a single-laboratory validation, or a multi-site collaborative study.

SMPRs are written and adopted by AOACI using the consensus of stakeholders representing the industry, government, and academic and/or research institutions. AOACI SMPRs are used by AOACI expert review panels (ERPs) in their evaluation of validation study data for method being considered for *Performance Tested Methods*^{SM} or AOACI *Official Methods of Analysis*^{SM} and can be used as acceptance criteria for verification at user laboratories.

1. Applicability

This document contains assessment parameters on the performance of Targeted Testing methods to monitor saffron powder (as a Spice) for the presence of the following potential economically motivated adulterants (EMAs): beet, pomegranate fibres, red dyed silk fibres, 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.

2. Analytical Technique

A Targeted Testing (TT) method(s) to monitor saffron powder for the presence of the following potential EMAs: beet, pomegranate fibres, red dyed silk fibres, 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.

A Targeted 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.

3. Definitions

Applicability Statement – This document contains assessment parameters on the performance of Targeted Testing methods to be used to monitor Saffron powder (Spice) for the presence of

the following potential economically motivated adulterants (EMA): beet, pomegranate fibres, red dyed silk fibres, 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.

Economically Motivated Adulteration – The fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller.

Saffron - It is the dried stigmas of *Crocus Sativas L*. It is cultivated in some regions of Asia (Kashmir, northern Iran), 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.

Each saffron flower has ONLY 3 stigmas which is used as a food additive due to its aroma, color and bitter taste and 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 of 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 **Protected Designations of Origin (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.

Authentic Saffron – Saffron quality is established by ISO based on aroma, bitterness and coloring strength. ISO standard ISO 3632-1:2011 establishes saffron quality as one that uses a UV/VIS spectrophotometric method that quantifies the flavour strength (expressed as the concentration of picrocrocin), the aroma strength (concentration of safranal) and the coloring strength (concentration of crocin).

Non-authentic substance or adulterant - A food item intentionally labelled 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 labelled as being cultivated in the PDO region in Spain can be used for substitution

It should be noted that the maximum limit of permissible colours 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 non-permitted food colour that include the synthetic dyes such as auramine, lead chromate, rhodamine, sudan-3, sudan-4, orange 2 and malachite green, are suspected to be mutagenic and carcinogenic and therefore present potentially serious health issues to the consumer.

Single Laboratory Validation – Demonstration by one laboratory of method performance on samples described according to internationally accepted validation guidelines contained in Guidance Documents such as AOAC'S Appendix D, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study" the ISO/IEC 17025:2017 Document: "General requirements for the competence of testing and calibration laboratories", the Codex Alimentarius Committee Guidance Document CAC/GL 71- 2009 - "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the use of Veterinary Drugs in Food Producing Animals" (Adopted 2009. Revision 2012, 2014); the "Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis CAC/GL-49-2003 "Harmonized Guidelines For Single-Laboratory Validation Of Methods Of Analysis"; "Guidelines on the use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative analysis of Residues CAC/GL 56-2005"; "Establishing the Fitness for Purpose of Mass Spectrometric methods." and SANTE/12682/2019. "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed"- A Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.

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 AOACI for classification as First Action Official Method of Analysis.

Multi-laboratory Validation – Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories according to guidelines described in the AOACI *Appendix D*, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study" be considered for classification as AOAC Final Action Method; "*Protocol for the design, conduct and interpretation of method performance studies*". Pure and Applied Chemistry, Horwitz, W. 1995. 67:331-343; "*Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food"- CAC/GL 27-1997*; "*Harmonized IUPAC Guidelines for the use of Recovery Information in Analytical Measurement" - CAC/GL 37-2001*; and "*Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement*"

The Predicted ($PRSD_R$) of REPRODUCIBILITY 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 –12 laboratories must be recruited to provide at least 8 valid data sets; two blind duplicate replicates at five concentration levels for each analyte/matrix combination to each collaborator.

HorRat (Repeatability, r) = $RSD_r/PRSD_R$

HorRat (Reproducibility, R) = $RSD_R/PRSD_R$

For Inter-laboratory studies: acceptable HorRat (R) of 1 with limits of acceptability of 0.5 to 2;

For Within-Laboratory studies: acceptable HorRat (r) of 0.3 – 1.3

4. Method Performance Requirements

Table 1: Method Performance Requirements for Colour and Non-colour Adulterants in Saffron

Analytical Parameter	**Acceptance Criteria for colour adulterants**	**Acceptance Criteria for colour 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

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks, and appropriate check standards.

6. 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.

7. 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 this SMPR.

c. For single lab validation studies, the method will be evaluated using prescribed adulterated materials as shown in Table 1 above. Methods approved at this level will proceed to a second level of evaluation (multi-laboratory) 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.

8. Maximum Time-to-Results

None.

References:

*CAC/GL 27-1997 - "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

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

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

CAC/GL 56-2005 – CXG 56 "*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/

Codex Alimentarius Committee Guidance Document CAC/GL 71- 2009 - "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme

Associated with the use of Veterinary Drugs in Food Producing Animals" (Adopted 2009. Revision 2012, 2014
http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

ISO/IEC 17025:2017 Guideline Document: "General requirements for the competence of testing and calibration laboratories", the *Codex Alimentarius Committee Guidance Document*
https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:en

SANTE/12682/2019. "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed"- A Guidance document on 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

Appendix D, "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Study" J. Assoc. Off. Anal. Chem. 72, 694–704(1989).

Appendix F: "Guidelines for Standard Method Performance Requirements"2016 AOAC Official Methods of Analysis

M. Thompson, S.L.R. Ellison and R. Wood, 2002. "*Harmonized Guidelines for Single-Laboratory Validation Of Methods Of Analysis*" Pure Appl. Chem., 74, (5) 835 – 855.
http://publications.iupac.org/pac/2002/pdf/7405x0835.pdf

Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P., and Stein, S. (2003). "*Establishing the Fitness for Purpose of Mass Spectrometric methods.*" Journal of the American Society for Mass Spectrometry 14: 528-541.

Horwitz, W. 1995 "*Protocol for the design, conduct and interpretation of method performance studies*". Pure and Applied Chemistry 67:331-343

Thompson, M., Ellison, S., Fajgelj, A., Willetts, P., & Wood, R. (1999) "*Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement*" Pure Applied Chemistry, 71: 337-348.