AOAC SMPR 2021.XXX; Draft AOAC Standard Method Performance Requirements (SMPRs) for Non-Targeted Testing (NTT) of Ingredients for Food Authenticity Methods Evaluation of Saffron

Intended Use

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, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for method being considered for *Performance Tested MethodsSM* or AOAC *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 Non-Targeted Testing methods to monitor the dried stigmas of *Crocus Sativas L* for the probable presence of Economically Motivated Adulterants (EMA).

This SMPR was designed to evaluate Non-Targeted Testing (NTT) methods developed to assess potential economic adulteration in saffron. The SMPR was purposely designed with general descriptions to be applicable to a broad range of innovative analytical platforms and chemometric approaches. Binary analytical results of "Authentic" or "Not Authentic" on defined samples from the performance of the method will be used to perform the evaluations by the Expert Review Panel.

Complete documentation of the collection and use of authentic samples is to be supplied by the method authors. The scope of authentic samples will be the applicable scope of the NTT method and expansion of the scope is possible with the inclusion of additional authentic samples into the baseline calibration, and validation using the protocol listed in this SMPR.

2. Analytical Technique

A non-targeted method to be used to evaluate foods and ingredients for possible EMAs. Any method generating a baseline fingerprint of the authentic material and comparing test sample fingerprints to assess differences will be considered. The final binary result identifies test samples as either authentic or potentially adulterated. This method demonstrates reliability using the requirements listed in this SMPR.

For single lab validation studies, the method will be evaluated using prescribed adulterated materials as shown in Tables 1a, 1b, and 1c. Methods may be validated using samples described in one, two, or all the tables. The applied table(s) will be used to define the scope of the analytical method. Methods approved at this level will proceed to a second level of evaluation (i.e., multi-laboratory validation) where blinded samples containing unknown adulterants will be sent to laboratories participating in the ensuing multi-laboratory validation.

The scope of the NTT method will be defined by the authentic samples used in generating the baseline fingerprint.

3. Definitions

Applicability Statement – a general statement about the intended purpose and scope of the method entailing key aspects of expected achievements for the specific situation and circumstances. Key

points to cover are the intended matrix scope, the purpose, and an indication of sensitivity, specificity, and significance (USP Appendix XVIII).

Authentic Samples – Samples representative of the genuine commodity. These samples should represent the food's or ingredient's variability seen naturally in the commodity. The authentic samples used to generate the product fingerprint will be used to properly define the NTT method testing scope.

Baseline Fingerprint – A food-specific model created by software evaluation of collected analytical data.

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 (USP Appendix XVIII).

Saffron - The dried stigmas of *Crocus sativus 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 $C_{16}H_{14}O$ and the bitter taste is produced by the monoterpene glucoside with molecular formula $C_{16}H_{26}O_7$. ISO 3632-1:2011 establishes saffron quality with spectrophotometric quantification of safranal and crocin.

Single Laboratory Validation – Demonstration by one laboratory of method performance on samples described in Tables 1a, 1b, and 1c.

Multilaboratory Validation – Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories.

Metanil Yellow – sodium 3-[4-anilinophenylazo] benzenesulfonate

Orange II – sodium 4-[(2*E*)-2-(2-oxonaphthalen-1-ylidene) hydrazinyl] benzenesulfonate

4. Method Performance Requirements

Methods may be validated using the samples described in one, two, or all of the following tables (Table 1a *Colorants*, Table 1b *Bulking Agents*, and/or Table 1c *Other Plants*). The applied table will be used to define the scope of the analytical method.

Table 1a: Method Performance Requirements for Saffron Adulterated with Colorants

Test	Adulterant	% Adulterant in Test Materials	Number of Samples to be Tested[1]	Number of Test Results Qualified as Adulterated
Baseline	None (Authentic Saffron)	0%	Establish Baseline Fingerprint[2]	
Validation using Authentic Samples[3]	None	0%	30	0
Validation[4]	Metanil Yellow CAS 587-98-4	1 ppm	30	30
Validation[4]	Orange II CAS 633-96-5	1 ppm	30	30
Validation[4]	Sudan 1 CAS 842-07-9	1 ppm	30	30
Validation[4]	Ponceau 4R CAS 2611-82-7	1 ppm	30	30
Validation[4]	Ponceau 6R CAS 2766-77-0	1 ppm	30	30

Table 1b: Method Performance Requirements for Saffron Adulterated with Bulking Agents

Test	Adulterant	% Adulterant in Test Materials	Number of Samples to be Tested[1]	Number of Test Results Qualified as Adulterated
Baseline	None (Authentic Saffron)	0%	Establish Baseline Fingerprint[2]	
Validation using Authentic Samples[3]	None	0%	30	0
Validation[4]	Pomegranate fibers	10%	30	30
Validation[4]	Beet	10%	30	30
Validation[4]	Gelatine fibers	10%	30	30
Validation[4]	Sandalwood	10%	30	30
Validation[4]	Campeche wood powder	10%	30	30
Validation[4]	Gardenia	10%	30	30
Validation[4]	Meat fibers	10%	30	30
Validation[4]	Starch	10%	30	30
Validation[4]	Glucose	10%	30	30
Validation[4]	Corn Silk	10%	30	30
Validation[4]	Red dyed silk fibers	10%	30	30

Table 1c: Method Performance Requirements for Saffron Adulterated with Other Plants

Test	Adulterant	% Adulterant in Test Materials	Number of Samples to be Tested[1]	Number of Test Results Qualified as Adulterated
Baseline	None (Authentic Saffron)	0%	Establish Baseline Fingerprint[2]	
Validation using Authentic Samples[3]	None	0%	30	0
Validation[4]	Safflower Sigma	10%	30	30
Validation[4]	Marigold Stigma	10%	30	30
Validation[4]	Dyed Corn Stigmas	10%	30	30
Validation[4]	Sandalwood	10%	30	30
Validation[4]	Campeche wood powder	10%	30	30
Validation[4]	Gardenia	10%	30	30
Validation[4]	Curcuma	10%	30	30

1. Multiple samples from the same batch of adulterated material can be used for method evaluation.
2. Full details on protocol used to establish an authentic fingerprint must be supplied.
3. Samples used for this step must be independent than those used to create the baseline and must cover the entire scope of the method.
4. Method validation using adulterated samples shall cover the entire scope used in creating the baseline fingerprint.

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks, and appropriate check standards.

6. Reference Materials

Detailed protocols used to identify reference materials as authentic and to create adulterated samples must be supplied.

7. Validation Guidance

a) Data demonstrating method performance is required.

b) Available guidance documents:

a. AOAC INTERNATIONAL Guidelines for Validation of Botanical Identification Methods, Journal of AOAC International Vol. 95, No. 1, 2012

b. Statistical analysis of interlaboratory studies. LII. Sample size needed to meet performance requirement on proportion. http://lcfltd.com/AOAC/tr347-SAIS-LII-sample-size-needed-for-PR-for-proportion.pdf

c. United States Pharmacopeia (USP). Appendix XVIII: Guidance on Developing and Validating Non-targeted Methods for Adulteration Detection. Food Chemicals Codex, 3rd supplement to 11th ed.; USP: Rockville, MD, 2019

8. Maximum Time-to-Results

None.