AOAC SMPR® 2021.012

Standard Method Performance Requirements (SMPRs®) for Nontargeted Testing (NTT) of Ingredients for Food Authenticity/Fraud Evaluation of Saffron

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 the 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.

2 Applicability

The document contains assessment parameters on the performance of nontargeted testing (NTT) methods to monitor the dried stigmas of *Crocus sativas* L. for the probable presence of economically motivated adulterants (EMA).

The SMPR was designed to evalute 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 an ERP.

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 the SMPR.

3 Analytical Technique

NTT 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. The method demonstrates reliability using the requirements listed in the SMPR.

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

4 Definitions

Applicability statement.—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 intended matrix scope, purpose, and 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. Authentic samples used to generate the product fingerprint will be used to properly define the NTT method testing scope.

Baseline fingerprint.—Food-specific model created by software evaluation of collected analytical data.

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 (USP Appendix XVIII).

Metanil yellow.—Sodium 3-[4-anilinophenylazo] benzenesulfonate.

Multilaboratory validation (MLV).—Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories.

Orange II.—Sodium 4-[(2*E*)-2-(2-oxonaphthalen-1-ylidene) hydrazinyl] benzenesulfonate.

Saffron.— 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* three stigmas and 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 $C_{16}H_{14}O$, 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 (SLV).—Demonstration by one laboratory of method performance on samples described in Table 1.

5 Method Performance Requirements

Methods may be validated using the samples described in Table 1 parts A–C (one, two, or all of the parts). The applied table will be used to define the scope of the analytical method.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks and appropriate check standards.

7 Reference Materials

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

8 Validation Guidance

(a) Data demonstrating method performance is required.

(b) For SLV studies, the method will be evaluated using prescribed adulterated materials as shown in Table 1. Methods may be validated using samples described in one, two, or all the parts of the table. The applied table 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., MLV), where blinded samples

Table 1. Method performance requirements

Test	Adulterant	Adulterant in test materials, %	No. of samples to be tested[a]	No. of test results qualified as adulterated
(A) Saffron adulterated with colorants				
Baseline	None (authentic saffron)	0	Establish baseline fingerprint[b]	
Validation using authentic samples[c]	None	0	30	0
Validation[d]	Metanil yellow CAS 587-98-4	1 ppm	30	30
Validation[d]	Orange II CAS 633-96-5	1 ppm	30	30
Validation[d]	Sudan 1 CAS 842-07-9	1 ppm	30	30
Validation[d]	Ponceau 4R CAS 2611-82-7	1 ppm	30	30
Validation[d]	Ponceau 6R CAS 2766-77-0	1 ppm	30	30
(B) Saffron adulterated with bulking agents				
Baseline	None (authentic saffron)	0	Establish baseline fingerprint[b]	
Validation using authentic samples[c]	None	0	30	0
Validation[d]	Pomegranate fibers	10	30	30
Validation[d]	Beet	10	30	30
Validation[d]	Gelatine fibers	10	30	30
Validation[d]	Sandalwood	10	30	30
Validation[d]	Campeche wood powder	10	30	30
Validation[d]	Gardenia	10	30	30
Validation[d]	Meat fibers	10	30	30
Validation[d]	Starch	10	30	30
Validation[d]	Glucose	10	30	30
Validation[d]	Corn silk	10	30	30
Validation[d]	Red dyed silk fibers	10	30	30
(C) Saffron adulterated with other plants				
Baseline	None (authentic saffron)	0	Establish baseline fingerprint[b]	
Validation using authentic samples[c]	None	0	30	0
Validation[d]	Safflower stigma	10	30	30
Validation[d]	Marigold stigma	10	30	30
Validation[d]	Dyed corn stigmas	10	30	30
Validation[d]	Sandalwood	10	30	30
Validation[d]	Campeche wood powder	10	30	30
Validation[d]	Gardenia	10	30	30
Validation[d]	*Curcuma*	10	30	30

[a] Multiple samples from the same batch of adulterated material can be used for method evaluation. Each sample must be analyzed separately.
[b] Full details on protocol used to establish an authentic fingerprint must be supplied.
[c] Samples used for this step must be independent than those used to create the baseline and must cover the entire scope of the method.
[d] Method validation using adulterated samples shall cover the entire scope used in creating the baseline fingerprint.

containing unknown adulterants will be sent to laboratories participating in the ensuing MLV.

(c) Available guidance documents:

(*1*) *AOAC INTERNATIONAL Guidelines for Validation of Botanical Identification Methods* (2012) *J. AOAC Int.* **95**, 268–272(2012); DOI: 10.5740/jaoacint.11-447

(*2*) *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

(*3*) U.S. Pharmacopeia (USP) (2019) *Appendix XVIII: Guidance on Developing and Validating Nontargeted Methods for Adulteration Detection, Food Chemicals Codex*, 3rd Supplement to 11th Ed., USP, Rockville, MD, USA

9 Maximum Time-to-Results

None.

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