Intended Use

AOAC 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℠ or AOACI Official Methods of Analysis℠ 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 monoterpenne with molecular formula
C₁₀H₁₄O and the bitter taste is produced by the monoterpenne glucoside with molecular formula
C₁₆H₂₆O₇

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.


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 (PRSDR) 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) = $\frac{RSD_r}{PRSD_R}$

HorRat (Reproducibility, R) = $\frac{RSD_R}{PRSD_R}$

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

<table>
<thead>
<tr>
<th>Analytical Parameter</th>
<th>Acceptance Criteria for colour adulterants</th>
<th>Acceptance Criteria for colour adulterants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>1 – 30 %</td>
<td>1 – 30 %</td>
</tr>
<tr>
<td>LOQ (%)</td>
<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>Recovery %</td>
<td>80 – 120 %</td>
<td>80 – 120 %</td>
</tr>
<tr>
<td>Accuracy %</td>
<td>± 20 %</td>
<td>± 20 %</td>
</tr>
<tr>
<td>Precision (Repeatability) RSD$_r$</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Precision (Reproducibility) RSD$_R$</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Beet, pomegranate fibers, red dyed silk fibers, safflower and marigold to red stigmas of saffron, dried 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:


Associated with the use of Veterinary Drugs in Food Producing Animals” (Adopted 2009. Revision 2012, 2014


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


