AOAC SMPR 2021.XXX; Draft AOAC Standard Method Performance Requirements (SMPRs) for Targeted Testing (TT) of Vanilla Adulterants
Version 2; May 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 vanilla (as a flavouring agent) for the presence of the following potential economically motivated adulterants (EMAs): p-coumaric acid, 4-hydroxybenzaldehyde, vanillic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, vanillyl alcohol, ferulic acid, piperonal and ethyl vanillin.

2. Analytical Technique

A Targeted Testing (TT) method(s) to monitor vanilla extract for the presence of the following potential EMAs: p-coumaric acid, 4-hydroxybenzaldehyde, vanillic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, vanillyl alcohol, ferulic acid, piperonal and ethyl vanillin.

A Targeted method to be used to monitor and enforce regulatory requirements for vanilla adulterants in food.

Any quantitative method capable of detecting, identifying and quantifying the presence of an adulterating ingredient in vanilla 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 vanilla extract for p-coumaric acid, 4-hydroxybenzaldehyde, vanillic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, vanillyl alcohol, ferulic acid, ethyl vanillin and piperonal as adulterants.

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.

Vanilla - vanillin is the primary flavour element in vanilla extract. Wood lignin and other bean-producing plants are its source. One of those sources is tonka bean extract. It smells and tastes just like vanilla and industry uses it as an aromatic for things like pipe tobacco. But, it also contains a compound called coumarin not found in real vanilla.

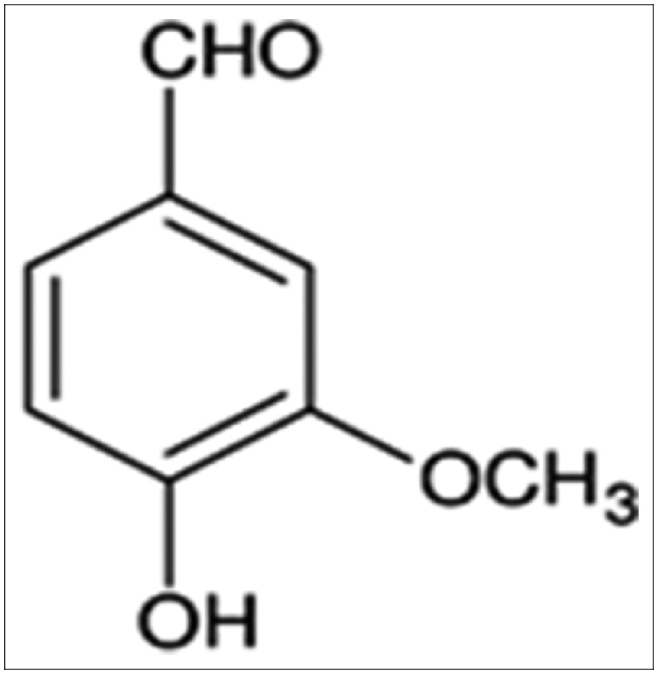
Pure Vanilla Extract (Ingredients: Water; Alcohol; Vanilla Bean Extractives)
Vanilla Extract is defined by the FDA in CFR 21, part 169 (1). It must be extracted from no less than 13.35 ounces of vanilla beans per gallon, in a minimum of 35% ethyl alcohol, with the remaining liquid being water. The addition of sugar, glycerin, or corn syrup is also allowed and is sometimes added to Vanilla Extract to mask the flavor of synthetic alcohol. Pure vanilla-bean extract is made by putting vanilla beans in a solution of ethyl alcohol and water. Its costly production and labor-intensive extraction process coupled with cyclones, drought and theft, have contributed to making this ingredient susceptible to food fraud.

Alcohol Free Pure Vanilla Flavor (Ingredients: Water; Glycerin; Vanilla Bean Extractives)
Most companies in the USA make this product with the same 13.35 ounces of vanilla beans as Vanilla Extract, but replace the alcohol with glycerin. According to FDA rules (1), because this product does not contain at least 35% alcohol, it cannot be called Vanilla Extract.

Natural Vanilla Flavor (Ingredients: Water; Alcohol; Glycerin; Vanilla bean Extractives; Botanical Extractives). Natural Vanilla Flavor is made with real vanilla beans and augmented with other plant extracts to approximate the flavor of pure vanilla extract. It doesn't have the flavor complexity of pure vanilla extract, but it makes a reasonable clean-label substitute for companies hoping to reduce costs amidst the currently high vanilla prices. The FDA does not define natural vanilla flavor so the amount of actual vanilla content will vary depending on the manufacturer. Natural vanilla is a complex mixture of flavor components extracted from the cured pods of different species of plant genus Vanilla: Vanillus planifolia and Vanillus tahitensis (Rao and Ravishankar 2000 (2)). However, V. planifolia is valued most because of its pod quality and yield. The fruity, floral fragrance of cured vanilla pods, combined with a deep, aromatic body, makes it a widely accepted flavoring agent. The active constituents of vanilla are responsible for its various biological and therapeutic activities.

The flavor profile of vanilla contains more than 200 components, of which only 26 occur in concentrations greater than 1 mg/kg. The aroma and flavor of vanilla extract is attributed mainly to the presence of vanillin (4-hydroxy-3-methoxybenzaldehyde; in the figure below), which

occurs in a concentration of 1.0 - 2.0 % w/w in cured vanilla pods (Westcott et al. 1994 (3); Bettazzi et al. 2006 (4); Sharma et al. 2006 (5)).



True vanilla pods possess a pure delicate spicy flavor that cannot be duplicated exactly by synthetic products and because of limited supply, natural vanilla is very expensive and, attracts numerous efforts to blend and adulterate. Also, the flavor quality of vanilla extracts vary considerably, depending upon the origin, curing technique used, storage conditions, extraction methods, and age of the vanilla extract itself. Green vanilla pods possess no flavor. The characteristic flavor and aroma of vanilla pods develops during the curing process in which enzymatic changes occur. The action of naturally induced β-glycosidases on the glycosides releases various vanilla flavor components. Curing process consists of four steps: scalding/killing, sunning/sweating, drying and conditioning/aging (Karas et al. 1972 (6); Havkin-Frenkel and Dorn 1996 (7); Dignum et al. 2002 (8)).

Artificial Vanilla (The ingredients vary but usually include Water; Vanillin derived from wood pulp; Synthetic Alcohol; Caramel coloring; Corn Syrup). There are plenty of fake vanilla products made in the USA that are safe for human consumption. The color of these products varies from clear to dark brown depending on the amount of food coloring added.

Production of vanillin

At present about 97% of vanillin sold in the market comes mainly from the synthetic sources using coniferin, eugenol, safrole, guaiacol (Bedoukian 1986 (9)) and lignin (Hearon and Lo 1980

(10); Wu et al. 1994 (11); Sande and Sears 1996 (12); Hocking 1997 (13); Bjørsvik 1999 (14); Qiang and Zhonghao 2001 (15); Kozlov and Gogotov 2001 (16)). Although vanillin produced by these means is able to meet the global annual demand, it suffers from serious drawbacks.

For one, the aroma of synthetically produced vanillin is not comparable with that of natural vanillin. Secondly, chemical synthesis involves use of hazardous chemicals (and hence under current US and European legislations cannot be used in natural flavours), resulting in decreased consumer appeal the world over. However, the production and isolation of vanillin from natural sources present an altogether different scenario. The reason behind it is the huge disparity in efforts put in and the yield per hectare. The cultivation of vanilla is a time-consuming and labour intensive process, yet the yield is not very high (Rao and Ravishankar 2000 (2)). Very few attempts have been reported for the isolation of natural vanillin from vanilla extract. [17: International Journal of Food Sciences and Nutrition, June 2008; 59(4): 299326. A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents ARUN K. SINHA, UPENDRA K. SHARMA, & NANDINI SHARMA]

Non-authentic substance or adulterant *- A food item intentionally labelled as vanilla when the product developer knows that another substance or an adulterant such as those listed in the applicability statement has been used to adulterate vanilla for economic gain.*

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" (18),* 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) (19); the "*Harmonized ISO/IUPAC/AOAC Guidelines for Single-Laboratory Validation of Methods of Analysis CAC/GL-49-2003 (20)* "*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 (21)*"; "*Establishing the Fitness for Purpose of Mass Spectrometric methods (22)."* and *SANTE/12682/2019 (23). "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*, (24) "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 (25); "*Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food"- CAC/GL 27-1997 (26)*; "*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"(27)*

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 labs 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 Vanilla Adulterants - P-coumaric acid, 4-hydroxybenzaldehyde, vanillic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, vanillyl alcohol, ferulic acid, ethyl vanillin and piperonal.

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	**1 - 50**
LOQ (%)	**≤1**
Recovery %	**80 - 120**
Accuracy %	**± 20**
Precision (Repeatability) RSD_r	**10**
Precision (Reproducibility) RSD_R	**18**

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.

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*27. 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