AOAC SMPR® 2021.016

Standard Method Performance Requirements (SMPRs®) for Targeted Testing (TT) of Vanilla Adulterants

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 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 targeted testing (TT) methods to monitor vanilla (as a flavoring 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.

3 Analytical Technique

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.

TT 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.

4 Definitions

Alcohol-free pure vanilla flavor.—Ingredients: water, glycerin, and vanilla bean extractives. Most companies in the United States make this product with the same 13.35 oz vanilla beans as vanilla extract but replace the alcohol with glycerin. According to U.S. Food and Drug Administration (FDA) rules (1), because this product does not contain at least 35% alcohol, it cannot be called vanilla extract.

Applicability statement.—Assessment parameters on the performance of TT 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.

Artificial vanilla.—Ingredients vary but usually include water, vanillin derived from wood pulp, synthetic alcohol, caramel coloring, and corn syrup. Plenty of fake vanilla products are made in the United States 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.

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.

Multilaboratory validation (MLV).—Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories according to guidelines described in OMA *Appendix D* (2) to be considered for classification as AOAC Final Action Method and in refs 3–5.

The predicted relative standard deviation of reproducibility ($PRSD_R$) 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 eight valid data sets, two blind duplicate replicates at five concentration levels for each analyte/matrix combination to each collaborator.

$$\text{HorRat (repeatability, r)} = RSD_r/PRSD_R$$

$$\text{HorRat (reproducibility, R)} = RSD_R/PRSD_R$$

For interlaboratory studies, acceptable HorRat (R) of 1 with limits of acceptability of 0.5–2; for within-laboratory studies, acceptable HorRat (r) of 0.3–1.3.

Natural vanilla flavor.—Ingredients: water, alcohol, glycerin, vanilla bean extractives, and 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 does not 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* (6). 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; *see* Figure 1), which occurs in a concentration of 1.0–2.0% (w/w) in cured vanilla pods (7–9).

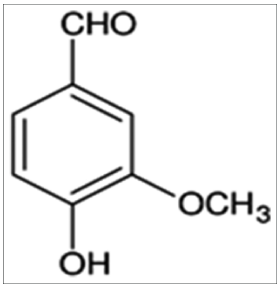

Figure 1. Chemical structure of vanillin (4-hydroxy-3-methoxybenzaldehyde).

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 develop 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 (10–12).

Nonauthentic substance or adulterant.—Food item intentionally labeled 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.

Production of vanillin.—At present about 97% of vanillin sold in the market comes mainly from the synthetic sources using coniferin, eugenol, safrole, guaiacol (13), and lignin (14–20). 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 U.S. and European legislations cannot be used in natural flavors), resulting in decreased consumer appeal the world over. However, 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 labor-intensive process, yet the yield is not very high (6). Very few attempts have been reported for the isolation of natural vanillin from vanilla extract (21).

Pure vanilla extract.—Ingredients: water, alcohol, and vanilla bean extractives. Vanilla extract is defined by FDA (1). It must be extracted from no less than 13.35 oz. 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 placing 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.

Single-laboratory validation (SLV).—Demonstration by one laboratory of method performance on samples described according to internationally accepted validation guidelines contained in guidance documents (2, 22–27).

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

Parameter	Acceptance criteria
Analytical range, %	1–50
LOQ, %	≤1
Recovery, %	80–120
Accuracy, %	±20
Precision (repeatability) RSD_r	10
Precision (reproducibility) RSD_R	18

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 AOAC INTERNATIONAL for adoption and publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* as First Action status.

Vanilla.—Vanillin is the primary flavor element in vanilla extract. Wood lignin and other bean-producing plants are its source. One of those sources is tonka bean extract, which smells and tastes like vanilla and used by industry as an aromatic for products such as pipe tobacco. But it also contains a compound called coumarin not found in real vanilla.

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks and appropriate check standards.

7 Reference Materials

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.

8 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 the SMPR.

(c) For SLV studies, the candidate method will be evaluated using prescribed adulterated materials as shown in Table 1. Methods approved at this level will proceed to a second level of evaluation (MLV), 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.

9 Maximum Time-to-Results

None.

10 References

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