Juul Labs®

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Electronic Submission

Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

Re: Docket No. FDA-2021-D-0756; Comment on Validation and Verification of Analytical Testing Methods Used for Tobacco Products; Draft Guidance for Industry

To whom it may concern:

Juul Labs, Inc. (JLI or the Company; we or our) appreciates the opportunity to provide comment to the Food and Drug Administration (FDA or the Agency) on its *Guidance for Industry (Draft): Validation and Verification of Analytical Testing Methods Used for Tobacco Products* (Draft Guidance).[1]

I. INTRODUCTION

JLI supports a transparent, predictable, and efficient regulatory framework to foster innovation of potentially less harmful alternatives for adult smokers. In turn, this regulatory framework can ensure a marketplace of fully regulated, scientifically-substantiated products that can reduce the death and disease associated with cigarette smoking. The Draft Guidance serves as another step in that direction. Standardizing the measurement of chemical constituents using analytical methods validated for critical attributes (accuracy, precision, specificity, detection and quantitation limits, linearity, and range) provides clarity on product testing, supports the development of new products, and adds efficiencies to the premarket review of such applications.

Traditional tobacco products are diverse and inherently complex. Combustible-cigarette smoke comprises over 7,000 individual chemical constituents,[2] and there are over 4,000 chemicals identified in smokeless tobacco products.[3]

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[1] 86 Fed. Reg. 72603 (Dec. 22, 2021).

[2] *See, e.g.*, Centers for Disease Control and Prevention, Chemicals in Tobacco Smoke, https://www.cdc.gov/tobacco/data_statistics/sgr/2010/consumer_booklet/chemicals_smoke/index.htm (last accessed Feb. 22, 2022).

[3] *See, e.g.*, FDA, Chemicals in Tobacco Products and Your Health, https://www.fda.gov/tobacco-products/health-effects-tobacco-use/chemicals-tobacco-products-and-your-health#references (last accessed Feb. 22, 2022).

More novel products, like electronic nicotine delivery systems (ENDS), generally are less chemically complex but can vary in design and formulation. E-liquids primarily comprise non-flavor ingredients in addition to nicotine, such as propylene glycol and glycerol that are used as carriers. Levels of these carriers, however, can vary significantly among different ENDS products. A study of fifty-four unique ENDS products purchased through online retailers in Germany revealed that levels of propylene glycol ranged between 0.3–95 /100 g of e-liquid and levels of glycerol ranged between 0.4–98 /100 g of e-liquid.[4] Beyond variability in primary ingredients, a recent analysis of the Dutch ENDS market identified 219 unique flavor ingredients used in e-liquids; the mean number of flavor ingredients in individual e-liquid formulations was ten, although some formulations included many more.[5]

Given the diversity and complexity of tobacco products, coupled with methodological challenges raised by differences in tobacco-product matrices,[6] our internal approach to validating analytical methods has followed the International Conference for Harmonization (ICH) guideline on the *Validation of Analytical Procedures: Text and Methodology Q2 (R1)* (ICH Guideline).[7] The ICH Guideline ensures rigor on method validation, while providing sufficient flexibility in light of differences in and across tobacco-product classes for testing and analytical purposes.

JLI's methods are validated using a range of products representing an array of test samples. Required validation elements — including accuracy, precision, limit of detection, limit of quantitation, selectivity, trapping efficacy, stability, and robustness — are evaluated in the context of a product matrix.[8] We do not separately validate matrix extraction and analytical measurement. This is because analytical-method performance is dependent upon matrix effects such as interferences, analyte responses, and impacts to analyte recovery. Because of these effects, we do not believe analytical methods for tobacco products should be developed outside the context of an intended target matrix. Following the ICH Guideline, this approach for validating analytical methods provides a

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[4] *See* J. Hahn, et al., Electronic Cigarettes: Overview of Chemical Composition and Exposure Estimation, Tobacco Induced Diseases (2014).

[5] *See* E. Krusemann, et al., Comprehensive Overview of Common E-liquid Ingredients and How They Can Be Used to Predict an E-liquid's Flavour Category, Tobacco Control (2021).

[6] For example, we consider tobacco-flavored e-liquids to be a product matrix. Minor differences in flavor ingredients among tobacco-flavored e-liquids do not impact the performance of our methods for measuring components and constituents. On the other hand, menthol flavored e-liquids represent a distinct product matrix. This is because the flavor ingredients in menthol e-liquids can alter the physical properties of the matrix relative to tobacco-flavored e-liquids.

[7] International Conference on Harmonization, Validation of Analytical Procedures: Text and Methodology (Q2(R1) (hereinafter, ICH Guideline), available at https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf.

[8] Additional examples of product matrices include ENDS aerosol generated under intense and non-intense puffing conditions and flavored and unflavored e-liquid formulations.

comprehensive demonstration that such analytical procedures are suitable, reliable, and fit for purpose.

In fact, other centers within FDA, including the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research, have incorporated the ICH Guideline in their *Guidance for Industry: Q2(R1) Validation of Analytical Procedures: Text and Methodology*, which they describe as "the same, in substance, as the November 2005 ICH Q2(R1) guideline."[9] And scientists and technical experts within the Center for Tobacco Products (CTP) have utilized the ICH Guideline to evaluate the quality of published analytical measurements for tobacco products. For example, in a recent study, CTP researchers reported on a comprehensive review of analytical studies for ENDS products to evaluate whether they "included adequate method validation characteristics in the publication for appropriate interpretation of data quality for informing tobacco regulatory science."[10] The researchers based their assessment of method-validation characteristic on the ICH Guideline for method validation — describing the ICH Guideline as "well-established and globally recognized for pharmaceutical industries."[11]

Building from the Draft Guidance, we believe that analytical methods validated in accord with the ICH Guideline are suitable and reliable to support regulatory submissions for tobacco products and satisfy regulatory-reporting requirements. We encourage the Agency to adopt such an approach and align more closely with the ICH Guideline in the final guidance.[12] In support of both this approach and on other issues, we provide technical comments and considerations for the Agency and requests for clarification on certain elements of the Draft Guidance.

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[9] FDA, Guidance for Industry: Q2(R1) Validation of Analytical Procedures: Text and Methodology (2005), available at https://www.fda.gov/media/152208/download.

[10] S. Reilly, et al., Method Validation Approaches for Analysis of Constituents in ENDS, Tobacco Regulatory Science (2020).

[11] *Id.*

[12] In particular, we raise whether an additional method verification procedure is needed once a method has been fully validated using matrix samples. For example, JLI would validate a method for combustible cigarettes using low-tar and high-tar matrix samples to cover the range of tar in cigarette samples to which the method may be applied. Under this approach, it would be unnecessary to perform additional method verifications for cigarettes in which the measured tar falls within the range covered by the method validation.

II. TECHNICAL COMMENTS, CONSIDERATIONS, AND CLARIFICATIONS

A. Definition of "Validation"

FDA states that "[v]alidation of an analytical method applies to a specific laboratory, for a specific tobacco product formulation, and equipment performing the analytical test method for an intended use over a reasonable period of time."[13]

JLI and its third-party laboratories typically validate an analytical method for a specific product matrix, such as tobacco- or menthol-flavored ENDS aerosol and flavored or unflavored e-liquids. Multiple, specific tobacco-product formulations may exist within a single tobacco-product matrix — a product matrix for tobacco-flavored e-liquids and another product matrix for menthol-flavored e-liquids. Because our methods are validated for product matrices, we would not typically conduct separate method validation for specific tobacco products that share the same matrix. This approach, following the ICH Guideline, removes unnecessary redundancies but also ensures appropriate validation of the analytical methods for testing across characteristically similar products within the matrix.

We request that FDA clarify whether "specific tobacco product formulation" refers to a specific tobacco-product matrix type (e.g., tobacco-flavored e-liquid) or if the Agency is proposing that analytical method validation be performed for each specific tobacco-product formulation (e.g., separate method validations for tobacco-flavored e-liquids that differ only in minor flavor ingredients). In that vein, we suggest that the definition of "validation" be clarified to: "Validation of an analytical method applies to a specific laboratory, for a *specific tobacco matrix type*, and equipment performing the analytical test method for an intended use over a reasonable period of time." This change would be consistent with the ICH Guideline and established practices of regulated industry and laboratories for validating analytical methods for product matrices rather than specific products. Because analytical methods can be validated for tobacco-product matrices, conducting an additional method validation for each "specific product formulation" within a product matrix would be a significant burden without improving the quality of analytical measurements.

B. Definition of "Verification"

FDA states "[v]erification is typically recommended following a change to one of the procedures in a method or a change to the tobacco product being tested."[14] It is unclear what is meant by "change to the tobacco product being tested."

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[13] FDA, Guidance for Industry (Draft): Validation and Verification of Analytical Testing Methods Used for Tobacco Products, 4 (2021) (hereinafter, Draft Guidance).

[14] *Id.* at 5.

We request that FDA clarify if it is recommending that methods be verified when applying an analytical method to a different product type (e.g., cigarette smoke versus ENDS aerosol) or if the method should be verified in response to any change to a tobacco product (e.g., a change in an ingredient level). As noted above, JLI validates its analytical methods in the context of a product matrix, which includes a demonstration that the method is insensitive to minor changes in the matrix such as changes in ingredient levels or addictions/deletions of ingredients at very low quantities in the formulation. Provided changes to the product do not result in physical changes to the matrix, we do not believe it is necessary to verify methods in response to minor product changes if sufficient method specificity has been demonstrated.

C. Considerations for the Validation of Accuracy and Precision

FDA recommends the use of seven or more replicates per concentration to provide sufficient data to evaluate the accuracy of an analytical method.

Here, the ICH Guideline recommends that "[a]ccuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure)."[15] We agree that the number of replicates must be sufficient to evaluate method accuracy. In our experience, however, measuring three replicates at a minimum of three concentrations is sufficient to validate method accuracy.[16]

JLI also validates the precision of its analytical methods consistent with the ICH Guideline that recommends conducting "a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each)" or "a minimum of 6 determinations at 100% of the test concentration" for repeatability.[17] We typically perform precision using five replicates over three analysis days at a specified concentration, yielding fifteen determinations. This procedure provides both interday and intraday precision and includes data for the evaluation of repeatability, intermediate precision, and reproducibility.

D. Considerations for Determining Total Error of Measurement

FDA recommends using probability functions, including error in accuracy and precision of the analytical method, to define pass/fail criteria based on 95% confidence intervals.

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[15] *See* ICH Guideline, at 10.

[16] *See e.g.,* A. Eldridge, et al., Investigation of Number of Replicate Measurements Required to Meet Cigarette Smoke Chemistry Regulatory Requirements Measured Under Canadian Intense Smoking Conditions, Regulatory Toxicology and Pharmacology (2019).

[17] *See* ICH Guideline, at 10.

Consistent with the ICH Guideline, JLI typically assesses accuracy and precision separately during method validation. We use control-chart measurements of process-control samples to calculate measurement uncertainty and then define as the total error of the method. This approach further aligns with ISO/IEC 17025, requiring laboratories to identify all contributions to measurement uncertainty (including from sampling) and monitor and evaluate the data to determine if they are outside the predefined criteria.[18] Use of control-chart data enables the evaluation not only of total method error during validation but also over time as the method is implemented.

E. Considerations for Determining the Linearity and Range of an Analytical Method

FDA states that to determine the linearity and range of an analytical method "it is generally accepted that no fewer than five replicates of a defined concentration of analyte solution at no fewer than five concentrations are to be used."[19]

JLI agrees, consistent with the ICH Guideline, that at least five replicates are needed to establish the linearity of an analytical method. But the ICH Guideline does not recommend conducting five replicate analyses at each concentration. Instead, we establish calibration curves on each day of a method validation, which provides several determinations of calibration linearity and thus demonstrates method linearity. We believe this approach is sufficiently robust and data-driven to support the linearity and range of an analytical method.

F. Considerations for Analytical Test Method Development

FDA recommends the use of a laboratory reagent blank to detect potential contamination during sample preparation and analysis. FDA states "[t]he analyte being measured should be absent or below the limit of detection in the laboratory reagent blank for the particular method used."[20]

But certain common laboratory contaminants cannot be avoided in the absolute. Examples include:

- Ammonia, benzene, and toluene are ubiquitous air pollutants and cannot be fully excluded from the laboratory atmosphere; and[21]

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[18] *See* International Organization for Standardization, General Requirements for the Competence of Testing and Calibration Laboratories (ISO/IEC Standard No. 17025) (2017), available at https://www.iso.org/standard/66912.html.

[19] Draft Guidance, at 11.

[20] *Id.* at 13.

[21] *See, e.g.,* T. Tomoaki, et al., Air Contamination of Therapeutic Drug Monitoring Assay Reagents Results in Falsely High Plasma Ammonia Levels, Annals of Clinical Biochemistry (2022); Environmental

- Formaldehyde and acetaldehyde, which are "significant analytical impurities . . . at high concentrations," in the 2,4-Dinitrophenylhydrazine (DNPH) reagent used in the sampling of certain carbonyl compounds.[22]

To account for these potential, real-world testing anomalies, JLI suggests that FDA's recommendation for analyte measurements in laboratory blanks be revised to: "The analyte being measured should be absent or *below the limit of quantification* in the laboratory reagent blank for the particular method used." In certain instances in which the measured analyte is a common laboratory contaminant and levels of the contaminant in the laboratory blank are at or slightly above the limit of quantification, it is acceptable to subtract the level in the blank from the levels measured in the analytical samples.[23] For example, the International Organization for Standardization (ISO) method for determining water content of cigarette smoke total particulate matter (TPM) specifies that levels of water measured in sample blanks be subtracted from the levels of water measured in the TPM.[24]

G. Considerations for Analytical Measurement Procedure

FDA recommends evaluating the robustness of an analytical method by "using a set of experiments intended to identify the boundaries of acceptable instrument setting adjustments that can be made without causing a change in the AMP."[25]

JLI and its third-party laboratories typically develop and validate analytical sampling and measurement procedures as a system, including matrix-matched samples. This is because analytical-method performance is dependent upon matrix effects, such as interferences, analyte responses, and impacts to analyte recovery. A modification of instrument procedures would not be applicable under this approach to method validation. Rather, consistent with the ICH Guideline, we typically evaluate the robustness of an analytical method by altering sample-extraction times and sample-preparation ratios and performing validation with multiple product-matrix formulations.

Protection Agency, Monitor Values Report – Hazardous Air Pollutants, https://www.epa.gov/outdoor-air-quality-data/monitor-values-report-hazardous-air-pollutants (last accessed Feb. 22, 2022).

[22] Environmental Protection Agency, Sampling for Formaldehyde and Other Carbonyl Compounds in Indoor Air (Method No. 0100), available at https://www.epa.gov/sites/default/files/2015-12/documents/0100.pdf.

[23] Eurachem, Blanks in Method Validation: Supplement to Eurachem Guide The Fitness for Purpose of Analytical Methods (2019), available at https://www.eurachem.org/images/stories/Guides/pdf/MV_Guide_Blanks_supplement_EN.pdf.

[24] International Organization for Standardization, Cigarettes — Determination of Water in Total Particulate Matter from the Mainstream Smoke — Part 1: Gas-chromatographic Method (Method No. 10362-1:2019) (2019), available at https://www.iso.org/standard/72630.html

[25] Draft Guidance, at 15.

H. Clarification on Analytical Test Method Validation Acceptance Criteria

FDA recommends using the Horwitz-Thompson equation to determine acceptance criteria for test method analytical validation.[26] The Draft Guidance then includes illustrative data to demonstrate the application of the Horwitz-Thompson equation.

JLI agrees that this approach is reasonable for analytes found at high concentrations and that have limited variability. If sample-to-sample variability in levels of a particular analyte exceeds overall method variability, however, it is unclear how the Horwitz-Thompson equation would apply. For example, we have reported differences in levels of formaldehyde in tobacco-flavored e-liquid aerosols generated under intense and non-intense puffing conditions that exceed the method's variability.[27] These results are indicative of differences in product performance under different puffing conditions, but do not implicate the underlying accuracy of the analytical method for formaldehyde. We request that FDA provide insight into its thinking on determining acceptance criteria for highly variable analytes.

III. CONCLUSION

High-quality data on chemical constituents are essential to support tobacco regulatory science and the development of new, potentially less harmful products to reduce cigarette-related death and disease. The quality of the data is further supported by clear guidance on method validation to ensure suitability and reliability and facilitate an efficient review of premarket applications for new products. The Draft Guidance lays out those foundational principles.

We believe, however, that FDA should consider aligning its guidance closer to the ICH Guideline for method validation. JLI, others in regulated industry, and major third-party laboratories have validated hundreds of analytical methods across tobacco products, frequently adhering to the ICH Guideline. Data generated under these validated analytical methods have supported numerous substantial equivalence submissions, premarket tobacco product applications, and modified risk tobacco product applications. Incorporating the ICH Guideline in the final guidance will ensure that ongoing and future product development and testing for regulatory submissions and reporting will continue to provide FDA with high-quality data across its tobacco-regulatory activities.

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[26] *See* Draft Guidance, at 18–19; W. Horwitz, The Variability of AOAC Methods of Analysis As Used in Analytical Chemistry, J. Assoc. Off. Anal. Chem. (1997).

[27] X. Chen, et al., Targeted Characterization of the Chemical Composition of JUUL Systems Aerosol and Comparison with 3R4F Reference Cigarettes and IQOS Heat Sticks, Separations (2021).

Respectfully submitted,