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3 **Standard Method Performance Requirements for Determination of Ethanol in Kombucha**

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6 **Intended Use:** Use by trained technicians in a laboratory for routine quality assurance testing.

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8 **1. Applicability:**

9 Determination of low levels of ethanol as expressed as alcohol by volume (ABV) in
10 kombucha.

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12 **2. Analytical Technique:**

13 Any analytical technique that meets the following method performance requirements is
14 acceptable.

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16 **3. Definitions:**

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18 **Alcohol by volume (%ABV)**

19 A standard measure of how much alcohol (ethanol) is contained in a given volume of an
20 alcoholic beverage (expressed as a volume percent).

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22 **Ethanol**

23 The 2-carbon alcohol with a molecular formula of $\text{CH}_3\text{CH}_2\text{OH}$. CAS Registry Number: 64-17-5

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25 **Kombucha**

26 Kombucha is a fermented, effervescent tea beverage made by adding a symbiotic culture of
27 bacteria and yeast (SCOBY) to a solution of tea and sugar, and may include other
28 ingredients.

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30 **Limit of Quantitation (LOQ)**

31 The minimum concentration which quantitative results may be obtained with 95%
32 confidence.

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34 **Repeatability**

35 Variation arising when all efforts are made to keep conditions constant by using the same
36 instrument and operator and repeating during a short time period. Expressed as the
37 repeatability standard deviation (SD_r); or % repeatability relative standard deviation
38 ($\% \text{RSD}_r$).

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40 **Reproducibility**

41 The standard deviation or relative standard deviation calculated from among-laboratory
42 data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative
43 standard deviation ($\% \text{RSD}_R$).

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45 **Recovery Factor**

46 The fraction or percentage of the analyte that is recovered when the test sample is analyzed
47 using the entire method.

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4. **Method Performance Requirements:**

Analytical range (% ABV)	0.1 to 2.8
Limit of Quantitation (LOQ) (% ABV)	≤ 0.05
Accuracy (% of mean spiked recovery over the range of the assay)	97 to 102
Repeatability (RSD _r) %	≤ 4
Reproducibility (RSD _R)%	≤ 6

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ABV= alcohol by volume

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5. **System suitability tests and/or analytical quality control:**

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

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6. **Reference Material(s):**

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NIST Standard Reference Material[®]

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2893 Ethanol-water solution (nom. 0.08 %)

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2894 Ethanol-water solution (nom. 0.1 %)

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2895 Ethanol-water solution (nom. 0.2 %)

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2896 Ethanol-water solution (nom. 0.3 %)

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2897 Ethanol-water solution (nom. 2 %)

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Sigma Aldrich

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459836 200 proof, anhydrous, ≥99.5% (Sigma-Aldrich)

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Cerilliant CRMs

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E-037 Ethanol-80 (5 ampoule multi-pack), 80 mg/dL

E-038 Ethanol-100 (5 ampoule multi-pack), 100 mg/dL

E-039 Ethanol-200 (5 ampoule multi-pack), 200 mg/dL

E-041 Ethanol-150 (10 ampoule multi-pack), 150 mg/dL

E-044 Ethanol-400 (5 ampoule multi-pack), 400 mg/dL

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LGC Standards

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BCR-651, beer at 0.505 % (v/v) ethanol.

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BCR-652, beer at 0.051 % (v/v) ethanol .

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Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F:

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Guidelines for Standard Method Performance Requirements, 19th Edition of the AOAC

77

INTERNATIONAL Official Methods of Analysis (2012). Available at:

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http://www.eoma.aoac.org/app_f.pdf

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82 **7. Validation Guidance:**

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84 [Appendix F](#): Guidelines for Standard Method Performance Requirements; 19th Edition of the
85 AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at:

86 http://www.eoma.aoac.org/app_f.pdf

87

88 [Appendix K](#): Guidelines for Dietary Supplements and Botanicals; 19th Edition of the AOAC
89 INTERNATIONAL Official Methods of Analysis (2012). Available on line at:

90 http://www.eoma.aoac.org/app_k.pdf

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93 **8. Maximum Time-To-Result:** None

DRAFT