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Standard Method Performance Requirements for in vitro Determination of Total Antioxidant Activity in Foods, Beverages, Food Ingredients, and Dietary Supplements

1 Applicability

In vitro methods for determination of total (hydrophilic and lipophilic) antioxidant activity in foods, beverages, food ingredients, and dietary supplements.

2 Analytical Technique

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

3 Definitions

Antioxidant activity.—The in vitro measurement of the total potential of a food, beverage, ingredient, or dietary supplement to inhibit or delay the oxidation of other compounds. Trolox activity will be used as the baseline unit of measurement to allow comparison between methods.

Limit of detection (LOD).—The minimum concentration of a substance that can be measured and reported with 95% confidence that the antioxidant activity is greater than zero, and is determined from analysis of a low level of an antioxidant in a given matrix containing the antioxidant.

Limit of quantitation (LOQ).—The minimum analyte concentration for which quantitative results may be obtained with 95% confidence.

Repeatability precision.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r), or % repeatability relative standard deviation ($\%RSD_r$).

Reproducibility.—The SD or RSD calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R) or % reproducibility relative standard deviation ($\%RSD_R$).

Recovery factor.—The fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

4 Method Performance Requirements

See Table 1.

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, and a protocol to demonstrate suitability.

Table 1. Method performance requirements[a]

Analytical range	400–400,000[b]	
Limit of detection (LOD)[c]	133[b]	
Limit of quantitation (LOQ)[d]	400[b]	
Repeatability (RSD_r)[e]	400[b]	8.6%
	200,000[b]	3.4%
	400,000[b]	3.1%
Recovery factor	90–110%	
Reproducibility (RSD_R)[f]	400[b]	12.9%
	200,000[b]	5.1%
	400,000[b]	4.36%

[a] Concentrations apply to (*1*) foods as purchased; (*2*) foods as to be consumed; (*3*) beverages as to be consumed; (*4*) ingredients as purchased; (*5*) supplements as purchased.

[b] Units expressed as µmol trolox equivalents per 100 g. Trolox activity will be used as the baseline to allow comparison between methods. *Note*: The stated ranges may be adjusted based on the mechanics of the analytical method.

[c] Limit of detection (LOD) = Minimum concentration of a substance that can be measured and reported with 95% confidence that the antioxidant activity is greater than zero. In this table, units are expressed as µmol trolox equivalents per 100 g. Trolox activity will be used as the baseline to allow comparison between methods. *Note*: The stated LOQ may be adjusted based on the mechanics of the analytical method.

[d] Limit of quantitation (LOQ) = Level at or above which quantitative results may be obtained with a 95% degree of confidence. In this table, units are expressed as umol trolox equivalents per 100 g. Trolox activity will be used as the baseline to allow comparison between methods. *Note*: The stated LOQ may be adjusted based on the mechanics of the analytical method.

[e] Expected repeatability is 2/3 of the Horwitz-predicted %reproducibility (*see* footnote f).

[f] Expected reproducibility is based on the Horwitz equation for the listed concentrations. The Horwitz-predicted $\%RSD_R$ is based on the mass equivalent of one hydrogen and one electron (one hydride equivalent), which is transferred from the sample to the measuring agent. Therefore, for every micromole of trolox reacted, 1×10^{-8} g hydride equivalent per gram of sample are transferred. Thus, a unit of measurement factor of 10^{-8} was used for calculation using the Horwitz equation. *Note*: This approach may be adjusted depending on the method measurement system.

6 Reference Material(s)

Certified reference materials are available and should be used as appropriate.

7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*SM.

8 Maximum Time-to-Result

No maximum time.

Approved by the AOAC Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM). Final Version Date: March 21, 2012. Revised March 13, 2013. Effective Date: March 14, 2013.