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***Standard Method Performance Requirements (SMPR®) for Determination of Folate* in Dietary Supplements**

Intended Use: Reference Method compliance with FDA Dietary Supplement cGMPs, or other relevant regulatory agencies.

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, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC method review experts, including expert review panels in their evaluation of validation study data for methods being considered for AOAC *Performance Tested Methods*^{SM}, *Reviewed and Recognized*^{SM}, or AOAC *Official Methods of Analysis*^{SM}, and can be used as acceptance criteria for verification at user laboratories.

1. Applicability

Determination of folate in the forms of supplemental folic acid (CAS 59-30-3) and/or 5-methyl-tetrahydrofolate (CAS 68792-52- 9), in all forms of dietary supplements (such as tablets, capsules, softgels, gelcaps, liquids, powders, chewable gels, or other forms) intended to be taken by mouth.

2. Analytical Technique

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

3. Definitions

Dietary Ingredients. —Vitamin, mineral, herb, or other botanical; an amino acid; a dietary substance for use by humans to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients. {United States Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}

Dietary Supplements. —A dietary supplement is a product for humans to be taken by mouth that contains a "dietary ingredient" intended to supplement the diet. Dietary supplements may be found in many forms such as tablets, capsules, gels, softgels, gelcaps, liquids, or powders.

Limit of Detection (LOD). —The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk.

Limit of Quantification (LOQ). —The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Repeatability. —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 (SDr); or % repeatability relative standard deviation (%RSDr).

Reproducibility. —The SD or RSD calculated from among- laboratory data; expressed as the reproducibility standard deviation (SDR), or % reproducibility relative standard deviation (%RSDR).

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

4. Method Performance Requirements

See Table 1a and 1b

Table 1a. Method performance requirements *(folic acid)*			
Analytical Range	50 – 1500ug/g		
Limit of detection(LOD)	15ug/g		
Limit of quantitation(LOQ)	50ug/g		
Range	< 100ug/g	100 – 1000ug/g	> 1000ug/g
Repeatability (RSDr, %)*	≤ 10 (≤12)	≤ 7 (≤9)	≤ 5 (≤7)
Recovery, %*	90 –110 (85-115)	95 – 105 (90 – 110)	97 – 103 (92 – 108)
Reproducibility(RSDR, %)*	≤ 15 (≤18)	≤ 10 (≤12)	≤ 7 (≤10)
* Parentheses indicate requirements for difficult matrices (i.e., gummies, chewables)			

Table 1b. Method performance requirements *(5-methyl-THF)*			
Analytical Range	50 – 1500ug/g		
Limit of detection(LOD)	15ug/g		
Limit of quantitation(LOQ)	50ug/g		
Range	< 100ug/g	100 – 1000ug/g	> 1000ug/g
Repeatability (RSDr, %)*	≤ 10 (≤12)	≤ 7 (≤9)	≤ 5 (≤7)
Recovery, %*	80 –110 (75-120)	85 – 115 (80 – 115)	90 – 110 (85 – 115)
Reproducibility(RSDR, %)*	≤ 15 (≤18)	≤ 10 (≤12)	≤ 7 (≤10)
* Parentheses indicate requirements for difficult matrices (i.e., gummies, chewables)			

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.

6. *Reference Material(s)*

National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) SRM 3280 and 3289 - Multivitamin/Multielement Tablets. The SRM 3252, a protein drink mix, with an assigned folic acid value is also one of the choice in the lack of the SRM 3280 and 3289.

7. Validation Guidance

Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf) Also at: J. AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447.

8. Maximum Time-to-Signal

No maximum time.

9. Reference

Refer to USP <1151> Pharmaceutical Dosage Forms for definitions (1151 PHARMACEUTICAL DOSAGE FORMS (usp.org)