

# AOAC SMPR 2014.005

## Biotin in Infant Formula and Adult/Pediatric Nutritional Formula

### STAKEHOLDER PANEL ON INFANT FORMULA AND ADULT NUTRITIONALS

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### Intended Use: Reference Method for Dispute Resolution

#### 1 Applicability

Determination of total biotin in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

#### 2 Analytical Technique

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

#### 3 Definitions

*Adult/pediatric formula*.—Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment [AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN); 2010], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

*d-Biotin*.—5-[(3aS,4S,6aR)-2-oxohexahydrothieno[3,4-d]imidazol-4-yl]pentanoic acid (see Figure 1).

*Infant formula*.—Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding (Codex Standard 72-1981) made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

*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 quantitation (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

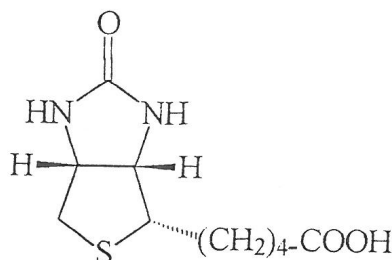


Figure 1. d-Biotin.

as the repeatability standard deviation ( $SD_r$ ); or % repeatability relative standard deviation (% $RSD_r$ ).

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

**Recovery.**—The fraction or percentage of spiked 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.

#### 6 Reference Material(s)

National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM) 1849a Infant/Adult Nutritional Formula or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849a consists of 10 packets, each containing approximately 10 g of material. Certified value of NIST 1849a is  $1.99 \pm 0.13$  mg/kg biotin.

#### 7 Validation Guidance

Recommended level of validation: *Official Methods of Analysis*<sup>SM</sup>.

#### 8 Maximum Time-to-Result

No maximum time.

**Table 1. Method performance requirements<sup>a</sup>**

Analytical range	0.1–150 <sup>b</sup>	
Limit of quantitation (LOQ)	≤0.1 <sup>b</sup>	
Repeatability ( $RSD_r$ )	0.1–1 <sup>b</sup>	≤8%
	>1 <sup>b</sup>	≤6%
Recovery	0.1–1 <sup>b</sup>	80 to 120% of mean spiked recovery over the range of the assay
	>1 <sup>b</sup>	90 to 110% of mean spiked recovery over the range of the assay
Reproducibility ( $RSD_R$ )	0.1–1 <sup>b</sup>	≤16%
	>1 <sup>b</sup>	≤12%

<sup>a</sup> Concentrations apply to (a) “ready-to-feed” liquids “as is”; (b) reconstituted powders (25 g into 200 g of water); and (c) liquid concentrates diluted 1:1 by weight.

<sup>b</sup> μg/100 g reconstituted final product.