NQAC
Nestlé Quality Assurance Center
Dublin

Technical Datasheet

Analysis Name: Total Amino Acids by AccQ-Tag & UHPLC-UV

Method Number: LI–00.594

Scope of Application: Soy- and milk-based infant and adult formula and similar materials, infant cereals, dry and wet pet foods.

Description: Proteins are hydrolyzed with 6 M HCl for 24 h at 110°C. Phenol (0.1%) is added to prevent halogenation of tyrosine. 3,3'-Dithiodipropionic acid (DDP) is added to convert cystine and cysteine to S-2-carboxyethylthiocysteine (XCys) as described by Barkholt & Jensen (1989). The resulting derivative can be separated from other amino acids and used for quantification of cystine (Cys2).

After hydrolysis and neutralization, amino acids and converted cysteine (XCys) are derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) using the AccQ•Tag Ultra Method (Waters Corporation, Milford, MA, USA). Derivatized amino acids are separated using reversed phase UHPLC with UV detection at 260 nm.

Sample Weight Required: 25 g

Method Reference: Barkholt V. and Jensen A. L. (1989). Amino acid analysis: Determination of cysteine plus half-cystine in proteins after hydrochloric acid hydrolysis with a disulphide compound as additive. Analytical Biochemistry 177, 318-322.

Waters Corporation (2007). UPLC amino acid analysis solution. System guide (71500129702/Revision B). Waters Corporation, Milford, MA, USA.

Analytical Platform: UPLC

Special Information: During acid hydrolysis, glutamine (Gln) and asparagine (Asn) are converted to glutamic acid (Glu) and aspartic acid (Asp), respectively. Thus, Glu values represent the combined values of Glu and Gln, and Asp values represent the combined values of Asp and Asn. Cys2 values represent the combined values of cysteine and cystine since both are converted to XCys by DDP.

Please provide estimate levels and notify us any time a sample is expected to have more than 10g/100g contribution from a single amino acid.

Analyte Reported	Alias	Unit	Limit of Quantification Range*	Reproducibility**
Histidine	HIS	g/100g	0.016 – 4.655	
Taurine (mg/100g)	Tau	mg/100g	12.516 – 3754.50	
Serine	SER	g/100g	0.011 – 3.153	
Arginine	ARG	g/100g	0.017 – 5.226	
Glycine	GLY	g/100g	0.008 – 2.525	
Aspartic Acid	ASP	g/100g	0.013 – 3.993	
Glutamic Acid	GLU	g/100g	0.015 – 4.414	
Threonine	THR	g/100g	0.012 – 3.574	
Alanine	ALA	g/100g	0.009 – 2.673	
Proline	PRO	g/100g	0.012 – 3.454	
Lysine	LYS	g/100g	0.015 – 4.386	
Tyrosine	TYR	g/100g	0.018 – 5.436	
Cystine	CYS	g/100g	0.024 – 0.961	
Methionine	MET	g/100g	0.015 – 4.476	
Valine	VAL	g/100g	0.012 – 3.515	
Isoleucine	ISO	g/100g	0.013 – 3.935	
Leucine	LEU	g/100g	0.013 – 3.935	
Phenylalanine	PHE	g/100g	0.017 – 4.956	

* LOQ varies depending on the matrix and sample aliquot taken for analysis

**Reproducibility: The relative difference between 2 independent single test results obtained using the same method, on identical test material by different operators at different days (for intermediate reproducibility test) should not be greater than:

- Infant formulas and infant cereals: 3 % to 16 % for all amino acids, except for Ser (up to 27%) and Cys2 (up to 39%)
- Dry and wet pet foods: 3% to 19% for all amino acids and 42% for Cys2

Which corresponds to the relative reproducibility limit, iR %, at 95% confidence level.