



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



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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.

Analyte Reported	Alias	Unit	Limit of Quantification Range*	Reproducibility**
Histidine	HIS	g/100g	0.0016 - 0.3103	
Taurine (mg/100g)	Tau	mg/100g	1.2516 - 250.32	
Serine	SER	g/100g	0.0011 - 0.2102	
Arginine	ARG	g/100g	0.0017 - 0.3484	
Glycine	GLY	g/100g	0.0008 - 0.1502	
Aspartic Acid	ASP	g/100g	0.0013 - 0.2640	
Glutamic Acid	GLU	g/100g	0.0015 - 0.2942	
Threonine	THR	g/100g	0.0012 - 0.2382	
Alanine	ALA	g/100g	0.0009 - 0.1782	
Proline	PRO	g/100g	0.0012 - 0.2302	
Lysine	LYS	g/100g	0.0015 - 0.2924	
Tyrosine	TYR	g/100g	0.0018 - 0.3624	
Cystine	CYS	g/100g	0.0012 - 0.2403	
Methionine	MET	g/100g	0.0015 - 0.2984	
Valine	VAL	g/100g	0.0012 - 0.2343	
Isoleucine	ISO	g/100g	0.0013 - 0.2623	
Leucine	LEU	g/100g	0.0013 - 0.2623	
Phenylalanine	PHE	g/100g	0.0017 - 0.3383	

* 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.