Hydrolysed Proteins

DOCUMENT M-CA, Section 4

ANALYTICAL METHODS

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
21/02/2020	Further information regarding the methods used for the determination of amino acids in CA 4.1.1 highlighted in yellow	Hydrolysed Proteins document M-CA 4

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 4 ANALYTICAL METHODS

CA 4.1 Methods used for the Generation of Pre-Approval Data

CA 4.1.1 Methods for the analysis of the active substance as manufactured

(a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

The total nitrogen and ammoniacal nitrogen have been determined by the method of Kjeldahl. Then, the content of organic nitrogen is calculated by the difference between the content of total nitrogen and the content of ammonium nitrogen. From that, the content of pure hydrolysed proteins is expressed by multiplying the organic nitrogen content by the conventional factor of 6.25

In response to the RMS request, further information regarding the methods used for the determination of amino acids have been provided under the form of a summary of the method PNT-M-109 used for the determination of amino acids.

The determination of primary and secondary amino acids was performed using an automated precolumn derivatization with OPA for primary amino acids and Fmoc for secondary amino acids, followed by a High-Performance Liquid Chromatography.

A schedule of accreditation for the laboratory involved in this study is also provided to argue that this laboratory is accredited by ENAC and that the used methods are based on the Official Methods established by the Spanish Real Decreto 1110/1991 Anexo num. 18.

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

No method was developed for the determination of relevant impurities in the active substance as manufactured since it does not contain relevant impurities of toxicological, ecotoxicological or environmental significance. For further information, please refer to Document J.

CA 4.1.2 Methods for risk assessment

According to the EFSA Journal 2012;10(2):2545, the requirement for methods of analysis for residues was waived as no residue definitions were proposed due to the nature of the Hydrolysed proteins.

Indeed, as demonstrated in Documents M-CA 5, M-CA 6, M-CA 7 and M-CA 8, Hydrolysed proteins are *per se* of low toxicity and not classified, and are therefore considered to pose a low risk to human health, to the environment and to non-target organisms. Furthermore, no maximum residue levels (MRLs) is required for the Hydrolysed proteins.

In this context, method for the risk assessment is not required, nor relevant.

(a) Methods In soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Not new data/method provided. Not required.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

Not new data/method provided. Not required.

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Not new data/method provided. Not required.

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

Not new data/method provided. Not required.

(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Not new data/method provided. Not required.

(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Not new data/method provided. Not required.

(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Not new data/method provided. Not required.

CA 4.2 Methods for Post-Approval Control and Monitoring Purposes

According to the EFSA Journal 2012;10(2):2545, the requirement for methods of analysis for residues was waived as no residue definitions were proposed due to the nature of the Hydrolysed proteins.

Indeed, as demonstrated in Documents M-CA 5, M-CA 6, M-CA 7 and M-CA 8, Hydrolysed proteins are *per se* of low toxicity and not classified and are considered to pose a low risk to human health, to the environment and to non-target organisms. Furthermore, no maximum residue levels (MRLs) is required for the Hydrolysed proteins.

In this context, method for the risk assessment is not required, nor relevant.

(a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

Not new data/method provided. Not required.

(b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

Not new data/method provided. Not required.

(c) Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Not new data/method provided. Not required.

(d) Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Not new data/method provided. Not required.