

# **Hydrolysed Proteins**

## **DOCUMENT M-CA, Section 2**

### **PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE**

## Version history (BIO)

Date	Data points containing amendments or additions and brief description	Document identifier and version number
2007-02-23	Initial Document J version, submitted for application of approval of the active substance.	J-Hydr.protein_BIOCEBO BIOIBERICA
2018-01-08	Analytical data of 5 batches of hydrolysed proteins in order to comply with Regulation 571/2012, which establishes that for the hydrolysed proteins some additional information is required regarding the specifications of the technical material, supported by the appropriate analytical data.	DOCUMENT J Hydrolysed proteins  CA 2 Analytical Profile of Batches (Former IIA 1.11 Analytical profile of batches)
2018-02-20	Physical and chemical properties determinations to fill the data requirements reported in EFSA review.	DOCUMENT J Hydrolysed proteins  CA 2 Physical and chemical properties (Former IIA 1.11 Analytical profile of batches)

<sup>1</sup> 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

## Table of Contents

<b>CA 2</b>	<b>PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE (BIO).....</b>	<b>5</b>
CA 2.1	Melting point, boiling point .....	5
CA 2.2	Vapour pressure, volatility .....	5
CA 2.3	Appearance (Physical state, colour) .....	5
CA 2.4	Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity .....	5
CA 2.5	Solubility in water .....	6
CA 2.6	Solubility in organic solvents .....	6
CA 2.7	Partition co-efficient n-octanol/water .....	7
CA 2.8	Dissociation in water .....	7
CA 2.9	Flammability and self-heating .....	7
CA 2.10	Flash point .....	7
CA 2.11	Explosive properties.....	8
CA 2.12	Surface tension .....	8
CA 2.13	Oxidising properties.....	8
CA 2.14	Other studies .....	8
<b>CA 2</b>	<b>PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE (PHY).....</b>	<b>10</b>
CA 2.1	Melting point, boiling point .....	10
CA 2.2	Vapour pressure, volatility .....	11
CA 2.3	Appearance (Physical state, colour) .....	12
CA 2.4	Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity .....	13
CA 2.5	Solubility in water .....	14
CA 2.6	Solubility in organic solvents .....	15
CA 2.7	Partition co-efficient n-octanol/water .....	15
CA 2.8	Dissociation in water .....	15
CA 2.9	Flammability and self-heating.....	16
CA 2.10	Flash point .....	17
CA 2.11	Explosive properties.....	18
CA 2.12	Surface tension .....	18
CA 2.13	Oxidising properties .....	19

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<b>CA 2.14</b>	<b>Other studies .....</b>	<b>19</b>
<b>CA 2</b>	<b>PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE (SIC) .....</b>	<b>20</b>
<b>CA 2.1</b>	<b>Melting point, boiling point .....</b>	<b>20</b>
<b>CA 2.2</b>	<b>Vapour pressure, volatility .....</b>	<b>20</b>
<b>CA 2.3</b>	<b>Appearance (Physical state, colour) .....</b>	<b>20</b>
<b>CA 2.4</b>	<b>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity .....</b>	<b>20</b>
<b>CA 2.5</b>	<b>Solubility in water .....</b>	<b>21</b>
<b>CA 2.6</b>	<b>Solubility in organic solvents .....</b>	<b>21</b>
<b>CA 2.7</b>	<b>Partition co-efficient n-octanol/water .....</b>	<b>21</b>
<b>CA 2.8</b>	<b>Dissociation in water .....</b>	<b>21</b>
<b>CA 2.9</b>	<b>Flammability and self-heating.....</b>	<b>22</b>
<b>CA 2.10</b>	<b>Flash point.....</b>	<b>22</b>
<b>CA 2.11</b>	<b>Explosive properties.....</b>	<b>22</b>
<b>CA 2.12</b>	<b>Surface tension .....</b>	<b>22</b>
<b>CA 2.13</b>	<b>Oxidising properties .....</b>	<b>22</b>
<b>CA 2.14</b>	<b>Other studies .....</b>	<b>22</b>

**CA 2      PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE (BIO)**

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.1    Melting point, boiling point</b>	Method: ASTM D56-2002	Hydrolysed proteins	102,3 °C	Y	Analytical data In LCA-Section 2
<b>CA 2.2    Vapour pressure, volatility</b>			Not applicable		
<b>CA 2.3    Appearance (Physical state, colour)</b>	Visual		Dark brown liquid		
<b>CA 2.4    Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</b>			IR spectra provided	Y	PhysChem analysis Hydrolysed proteins, Annex

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.5 Solubility in water</b>	Method: PNT-M-432		Very soluble Residues after 5 h: 0,23 % Residues after 24 h: <0,05 %	Y	Analytical data In LCA-Section 2
<b>CA 2.6 Solubility in organic solvents</b>			99,5 % soluble in acetone at 15°C (100 g/l) 99,5 % soluble in acetonitrile at 15°C (100 g/l) 99,8 % soluble in cyclohexane at 15°C (100 g/l) 99,8 % soluble in dichloromethane at 15°C (100 g/l) 84,2 % soluble in methane at 15°C (100 g/l)	Y	PhysChem analysis Hydrolysed proteins

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.7 Partition coefficient n-octanol/water</b>	Method: PNT-M-474 (OECD 107)		-1,7657	Y	Analytical data In LCA-Section 2
<b>CA 2.8 Dissociation in water</b> <ul style="list-style-type: none"> <li>dissociation constant(s) (pKa values)</li> <li>identity of dissociated species</li> <li>dissociation constant(s) (pKa values) of the active principle</li> </ul>			Not applicable		
<b>CA 2.9 Flammability and self-heating</b>			Not applicable		
<b>CA 2.10 Flash point</b>	UNE-EN 2719/03		>80 ° C	Y	18013479.01 In LCA-Section 2

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.11 Explosive properties</b>			Not applicable		
<b>CA 2.12 Surface tension</b>			41,9 mM/m at 20°C	Y	PhysChem analysis Hydrolysed proteins
<b>CA 2.13 Oxidising properties</b>			Not applicable		
<b>CA 2.14 Other studies</b>					

EFSA review report stated that no data were available on physical and chemical properties for the active substance from Bioiberica (Annex IIA, point 2). We provide new 5-batch analyses data and other laboratory analyses to fill the gap and complete the table above as much as possible. The complete laboratory reports are in the corresponding LC-A Section 2 folder.



Regulation 571/2012, amending Regulation (EU) No 540/2011 as regards to the conditions of approval of the active substances aluminium silicate, hydrolysed proteins and 1,4-diaminobutane (putrescine), establishes that for the hydrolysed proteins some additional information is required regarding the specifications of the technical material, supported by the appropriate analytical data. Moreover, in this mentioned Regulation, it is also stated that applicants shall submit the information requested to the European Commission through the Rapporteur European Member State (Greece in this case), by 1st of May 2013.

BIOIBERICA, as one of the applicants for the inclusion of Hydrolysed proteins in Annex I prepared a complete report performed in an external laboratory, Laboratory Munuera (Murcia, Spain), about the Analytical Profile (5 batch analysis) of the product “TECHNICAL BIOCEBO”, which is composed only by hydrolysed proteins ( $\geq 35.0$  % w/w).

This information was not provided when submitting the application for the inclusion of the active substance.

See analytical data of 5 batches of hydrolysed proteins.

<b>Parameter</b>	<b>Batch 12/0026</b>	<b>Batch 12/0027</b>	<b>Batch 23/0028</b>	<b>Batch 23/0029</b>	<b>Batch 23/0030</b>
Organoleptic characteristics	Dark brown liquid	Dark brown liquid	Dark brown liquid	Dark brown liquid	Dark brown liquid
Direct pH	4.48	4.55	4.56	4.59	5.12
Density	1.22 g/ml	1.22 g/ml	1.23 g/ml	1.22 g/ml	1.21 g/ml
Hydrolysed protein content	40.47 % w/w	42.60 % w/w	39.60 % w/w	41.84 % w/w	44.00 % w/w

The complete report is located in the J-Hydr.protein directory.

## CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE (PHY)

In first inclusion of hydrolysed protein DACONA (registered in Spain) was the representative ppp because it was the registered ppp with the higher content of beet molasses and the lower content of urea. In this renewal we replaced DACONA with ENTOMELA 50SL as the representative ppp because this is now the registered ppp with the same characteristics means the registered ppp with the highest beet molasses protein content.

As in case of Hydrolysed proteins the a.s. and the ppp are identical, the physical and chemical properties of a.s. are similar to the properties of formulated product. For more detailed information refer on formulation process on Document J - PHY and for detailed physical and chemical properties refer to MC-P Section 2 – ENT50.

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.1 Melting point, boiling point</b>	A1	Urea powder	<p>Hydrolysed protein is a mixture identical to the representative (ppp) formulation and not a purified active substance. So the determination of melting point or boiling point is not a physicochemical characteristic which can declare the purity of the a.s.</p> <p>For urea the melting point was determined to be 134°C.</p>		<p>Pfeiffer S. 2009 Urea 204611.03 Report.pdf</p>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.2 Vapour pressure, volatility</b>			<p>The vapor pressure only for urea was calculated on 20°C according to the equations and values referred on 6 published studies.</p> <p>1) <math>8.8 \times 10^{-4}</math> Pa at 20°C by Suzuki et al, 1955, Jones 1960</p> <p>2) <math>1.7 \times 10^{-4}</math> Pa at 20°C by Ferro et al 1987</p> <p>3) <math>4.9 \times 10^{-4}</math> at 20°C by Krasulin et al 1987</p> <p>4) <math>5.4 \times 10^{-4}</math> Pa at 20°C by Zaitsau et el 2003</p> <p>5) <math>7.5 \times 10^{-4}</math> at 20°C by Bernard et al 2011</p> <p>By extrapolation on 25oC according Suzuki et al, 1955 and Jones 1960 we get the values:</p> <p><math>1.2 \times 10^{-5}</math> mmHg or <math>1.6 \times 10^{-3}</math> Pa which are the values referred by EPA, EPIWIN IUCLID DATA SET, and ECHA site and used on urea DAR 2008.</p>		<p>Suzuki et al, 1955</p> <p>Jones 1960</p> <p>Ferro et al 1987</p> <p>Krasulin et al 1987</p> <p>Zaitsau et el 2003</p> <p>Bernard et al 2011</p>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.3 Appearance (Physical state, colour)</b>	Macroscopic examination	ENTOMELA 50SL  Batch No 5013011 Batch No 5013012 Batch No 5013013 Batch No 5013014 Batch No 5013015	<i>Dark Brown Liquid with characteristic odour</i>	N	HELLASCHEM 5 BATCHES ANALYSIS OF ENTOMELA 50SL  Iliopulos N. 2013 Certificate of analysis, 1320.130731/309  Iliopulos N. 2013 Certificate of analysis, 1321.130731/309  Iliopulos N. 2013 Certificate of analysis, 1322.130731/309  Iliopulos N. 2013 Certificate of analysis, 1323.130731/309  Iliopulos N. 2013 Certificate of analysis, 1324.130731/309

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</b>			<p>Hydrolysed protein is a mixture identical to the representative (ppp) formulation and not a purified active substance. So the spectral data cannot used as criterion of the purity of the a.s..</p> <p>Only for urea we are giving below the spectral data</p> <p>UV/VIS:  <a href="http://webbook.nist.gov/cgi/cbook.cgi?ID=C57136&amp;Units=CAL&amp;Mask=400#UV-Vis-Spec">http://webbook.nist.gov/cgi/cbook.cgi?ID=C57136&amp;Units=CAL&amp;Mask=400#UV-Vis-Spec</a></p> <p>IR:  <a href="http://webbook.nist.gov/cgi/cbook.cgi?ID=C57136&amp;Units=CAL&amp;Mask=80#IR-Spec">http://webbook.nist.gov/cgi/cbook.cgi?ID=C57136&amp;Units=CAL&amp;Mask=80#IR-Spec</a></p> <p>NMR:  <a href="http://sdb.sdb.aist.go.jp/sdb/cgi-bin/direct_frame_disp.cgi?sdbno=2958">http://sdb.sdb.aist.go.jp/sdb/cgi-bin/direct_frame_disp.cgi?sdbno=2958</a></p> <p>MS:  <a href="http://webbook.nist.gov/cgi/cbook.cgi?ID=C57136&amp;Units=CAL&amp;Mask=200#Mass-Spec">http://webbook.nist.gov/cgi/cbook.cgi?ID=C57136&amp;Units=CAL&amp;Mask=200#Mass-Spec</a></p>		<p>NIST DATABASE ONLINE UC-VIS-UREA DOC</p> <p>NIST DATABASE ONLINE</p> <p>Spectral Database for Organic Compounds SDBS – AIST</p> <p>NIST DATABASE ONLINE</p>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.5 Solubility in water</b>	5)Modified CIPAC MT.10.2	ENTOMELA 50SL Batch No 5013011 Batch No 5013012 Batch No 5013013 Batch No 5013014 Batch No 5013015	“Total insoluble matter: max 0.7%  The active substance hydrolysed proteins as Beet molasses urea hydrolysate was found to be soluble in water at a minimum percentage of 99.3%”	N	HELLASCHEM 5 BATCHES ANALYSIS OF ENTOMELA 50SL  Iliopulos N. 2013 Certificate of analysis, 1320.130731/309  Iliopulos N. 2013 Certificate of analysis, 1321.130731/309  Iliopulos N. 2013 Certificate of analysis, 1322.130731/309  Iliopulos N. 2013 Certificate of analysis, 1323.130731/309  Iliopulos N. 2013 Certificate of analysis, 1324.130731/309
	A6	Urea Powder	The water solubility of Urea was determined to be 624 g/l at 20 °C.		Pfeiffer S. 2009  Urea 204611.03 Report.pdf

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.6 Solubility in organic solvents</b>		For urea:	a) 24.11g/100g in methanol at 24.7oC b) 5.84 g/100g in ethanol at 25.5oC, c) 3.17 g/100g in isopropanol at 25.3oC		Fu-Ming Lee 1972
<b>CA 2.7 Partition coefficient n-octanol/water</b>	A8	Urea powder	The partition coefficient only for Urea was determined to be < -1.73 (sd: 0.16) at 22 ± 2°C.		Pfeiffer S. 2009 Urea 204611.03 Report.pdf
<b>CA 2.8 Dissociation in water</b> <ul style="list-style-type: none"> <li>dissociation constant(s) (pKa values)</li> <li>identity of dissociated species</li> <li>dissociation constant(s) (pKa values) of the active principle</li> </ul>			Not applicable		

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.9 Flammability and self-heating</b>	UNE-EN 2719/03  Scientifically justified statement. – Preliminary tests. A preliminary test was performed in correspondence with the Pendky-Martens closed tester method. The temperature of the sample was gradually increased up to boiling point.	ENTOMELA 50SL  Batch No 5014016	All known information on flammability of the ingredients (urea, beet molasses and water) and preliminary test proving that the preparation ENTOMELA 50SL can not be flammable and flash point cannot be determined.  No indication of ignition was observed.	N	JUSTIFIED STATEMENT FOR SAFETY PROPERTIES OF BEET MOLASSES UREA HYDROLYSATES  Iliopulos N. 2014 Certificate of analysis, 2342(1).140903/309
	A10	Urea powder	For urea: Flammability: Not highly flammable		Pfeiffer S. 2009 Urea 204611.03 Report.pdf



Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.10 Flash point</b>	Scientifically justified statement. – Preliminary tests. A preliminary test was performed in correspondence with the Pendky-Martens closed tester method. The temperature of the sample was gradually increased up to boiling point.	ENTOMELA 50SL Batch No 5014016	All known information on flammability of the ingredients (urea, beet molasses and water) and preliminary test proving that the preparation ENTOMELA 50SL can not be flammable and flash point cannot be determined.  No indication of ignition was observed.	N	JUSTIFIED STATEMENT FOR SAFETY PROPERTIES OF BEET MOLASSES UREA HYDROLYSATES  Iliopulos N. 2014 Certificate of analysis, 2342(1).140903/309
	A16	Urea powder	Self-ignition: No self-ignition below the melting point		Pfeiffer S. 2009 Urea 204611.03 Report.pdf

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.11 Explosive properties</b>	Scientifically justified statement. –	ENTOMELA 50SL Batch No 5014016	All known information on ingredients (urea, beet molasses and water) proving that the preparation ENTOMELA 50SL can not be explosive.  The formulation is not anticipated to have explosive properties.	N	JUSTIFIED STATEMENT FOR SAFETY PROPERTIES OF BEET MOLASSES UREA HYDROLYSATES  Iliopulos N. 2014 Certificate of analysis, 2342(1).140903/309
<b>CA 2.12 Surface tension</b>	A5. EC reg. 440/2008	ENTOMELA 50SL Batch No 5014016	70.6 (mN/m), 20o C, 1g/L Deionized water was used.	N	NEW (2014) PHYSICOCHEMICAL DATA ABOUT ENTOMELA 50SL  Iliopulos N. 2014 Certificate of analysis, 2342(1).140903/309

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.13 Oxidising properties</b>	Scientifically justified statement. –	ENTOMELA 50SL Batch No 5014016	All known information on ingredients (urea, beet molasses and water) proving that the preparation ENTOMELA 50SL can not be an oxidising agent.  The formulation is not anticipated to have oxidising properties.	N	JUSTIFIED STATEMENT FOR SAFETY PROPERTIES OF BEET MOLASSES UREA HYDROLYSATES  Iliopulos N. 2014 Certificate of analysis, 2342(1).140903/309
<b>CA 2.14 Other studies</b>					

## CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE (SIC)

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.1 Melting point, boiling point</b>	SICIT CHEMITECH S.p.A. Internal method		Confidential	N	
<b>CA 2.2 Vapour pressure, volatility</b>					
<b>CA 2.3 Appearance (Physical state, colour)</b>	SICIT CHEMITECH S.p.A. Internal method		Confidential	N	
<b>CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</b>	Spectrum One Perkin Elmer Reference Manual		Confidential	N	

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.5 Solubility in water</b>	SICIT CHEMITECH S.p.A. Internal method		Confidential		
<b>CA 2.6 Solubility in organic solvents</b>	SICIT CHEMITECH S.p.A. Internal method		Confidential	N	
<b>CA 2.7 Partition co-efficient n-octanol/water</b>					
<b>CA 2.8 Dissociation in water</b> <ul style="list-style-type: none"> <li>dissociation constant(s) (pKa values)</li> <li>identity of dissociated species</li> <li>dissociation constant(s) (pKa values) of the active principle</li> </ul>					

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
<b>CA 2.9 Flammability and self-heating</b>					
<b>CA 2.10 Flash point</b>					
<b>CA 2.11 Explosive properties</b>					
<b>CA 2.12 Surface tension</b>	Kruss Ring Method	Protein hydrolysate 55%w/w dry substance	Confidential	N	
<b>CA 2.13 Oxidising properties</b>					
<b>CA 2.14 Other studies</b>					