SOLUBILITY IN ORGANIC SOLVENTS AND IR SPECTRUM OF HYDROLYSED PROTEINS

Contract N.: M3O8PH190109-01

Sponsor: SICIT 2000 S.p.A.
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Study Monitor: SICIT 2000 S.p.A.
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Test item: Hydrolysed proteins

Study Director: Antonella Squarcia Released on: July 11th 2019
(Antonella Squarcia)

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development , Paris 1998.

- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE e 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).

- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments.

- GLP Provisional Certificate released by the Italian Ministry of Health on May 21st 2019 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affect the quality or integrity of the study.

Antonella Squarcia
Study Director
(Antonella Squarcia)

July 11th 2019
Date

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved Study Plan and the Standard Operating Procedures of Eurofins Biolab S.r.l.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

QAU INSPECTIONS	
PHASE	**DATE**
Experimentation:	
-Audit process-based	September,16th 2016
-Audit study-based	//
Documentation:	
- Study plan	April 08th, 2019
- Raw data	July 01st, July 11th, 2019
- Final report	July 01st,July 11th, 2019

This report accurately reflects the raw data.

Giovanna Patrizia Bianco

QA GLP
(Giovanna Patrizia Bianco)

July 11th 2019

Date

SUMMARY

The objective of this study is to estimate the solubility in organic solvents of test item "hydrolysed proteins" (aminoacids and peptide mixture in aqueous solution) according to CIPAC H, MT181 and the determination of its IR absorption spectrum.

The solubility of a substance is specified by the saturation concentration of the substance in a given solvent at a given temperature.
The solubility was determined by adding successive measured volumes of solvents to a known mass of test substance until complete dissolution.
The solvents with different polarities used for the test were: Heptane, Xylene, 1,2-Dichloroethane, Methanol, Acetone, Ethyl acetate.
The test was carried out at 20 °C.
The study showed that the solubility was < 10 g/L in each solvent.

The IR absorption spectrum was performed using IR spectroscopy with ATR (Attenuated Total Reflectance) in the scan range was 4000-500 cm^{-1}.

"Results" section reports the values obtained in detailed tables.

INTRODUCTION

This study has been carried out at the Test Facility Eurofins Biolab S.r.l. on behalf of the Sponsor on the test item.

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Solubility in Organic Solvents	April 09th, 2019	April 11th, 2019	L. D'Annunzio
IR absorption spectrum	April 09th, 2019	April 11th, 2019	L. D'Annunzio

BIBLIOGRAPHY

1. ECHA Guidance on the Biocidal Products Regulation, Volume I: Identity of the active substance/physico-chemical properties/analytical methodology – Information Requirements, Evaluation and Assessment. Parts A+B+C, Version 2.0, May 2018 (Reference: ECHA-18-G-03-EN).
2. CIPAC handbook H – MT 181 – Solubility in organic solvents

FILING

The Study Plan, the Final Report, amendments (if present) and all raw data are filed in the archives of Eurofins Biolab S.r.l. for 10 years after the issuing of the Final Report.
At the end of the study residual sample will be kept until the expiry date provided by the Sponsor.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the documents/products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the Test Facility Eurofins Biolab S.r.l.

TEST ITEM
(see annex #1)

Name	HYDROLYSED PROTEINS
Code	Not provided
Stability	5 years
Nature of the test substance	Raw Material/Chemical
Application for use	Co-formulant for Plant Protection Products
Application area	Agricolture
Hazard information	No hazard indications
Active Ingredient	NA

Active ingredient:
CAS number: 9015-54-7
Common name: Hydrolysed Proteins
Molecular formula: Unspecified
Structure: Unspecified
Waste procedure: the tested samples was discarded after the analytical tests according to local current laws.

ANALYSED SAMPLE
The sample analysed, representative of the test item, consists of a brown liquid contained in a white opaque plastic tank (HDPE) closed by a plastic screw cap.

Batch	19070125
Manufacturing date	February 18th, 2019
Expiry date	February 18th, 2024
Receiving number	IP-LV-2019079-ACV
Receiving date	March 20th, 2019
#ID (Eurofins Biolab)	LV-MAT-FOV7-19-079-0144:a
Storage until the beginning of the study	Room temperature

The test item and the information concerning the test item were provided by the Sponsor. All data related to the test item are under the responsibility of the Sponsor and have not been verified by the test facility.
Additional specifications, provided by the Sponsor, can be part of the report and do not require an amendment to study Plan.

EXPERIMENTAL REPORT- SOLUBILITY IN ORGANIC SOLVENTS

TEST METHOD
CIPAC method (Handbook H, MT181)

EXPERIMENTAL PROCEDURE
The solubility – within predefined ranges – of a test substance in organic solvents is determined by adding successive measured volumes of solvent to a known mass of test substance until complete dissolution. This method is not suitable for the determination of solubilities below 10 g/L.

A preliminary test was employed to determine the approximate solubility of the test item. The results of the preliminary test was used to select the most appropriate mass of test substance for the test.
The solvents for the test were:

- aliphatic hydrocarbon: Heptane
- aromatic hydrocarbon: Xylene
- halogenated hydrocarbon: *1,2*-Dichloroethane
- alcohol: Methanol
- ketone Acetone
- ester Ethyl acetate

The test was carried out at 20° (±1)° C.

REAGENTS
The solubility of test item was determined in solvents with different polarities.
The chosen solvents are of high purity analytical grade. The validity of reagents was checked before starting the analyses.

- Methanol (VWR, code 83662.320, lot 18Z0428)
- Heptane (VWR, code 24539.290, lot 17I014032)
- Xylene (Sigma-Aldrich, code 95672, lot STBF6548V)
- *1,2*-Dichloroethane (VWR, code 23343.294, lot 16H024003)
- Acetone (VWR, code 20066.321, lot 18B084009)
- Ethyl acetate (VWR, code 836660.290, lot 16Z1904)

EQUIPMENT
- Volumetric flask 10 mL – class A
- Thermostatic Bath (Lab. Companion, S/N Z055192) (B1911)

This instrument is qualified every year according to internal procedures.

EXPERIMENTAL DESIGN

Preliminary Test
To 0.1 g of test item in a volumetric flask (10 mL) increasing measured volumes of solvent were added according to steps below.

Total volume required for complete dissolution of 0.1 g (mL)	0.5	1.0	2.0	3.0	4.0	5.0	10.0
Approximate solubility (g/L)	>200	100-200	50-100	33-50	25-33	20-25	10-20

After each addition of solvent, the mixture was shaken vigorously and then stored at the test temperature with intermittent shaking for 10 min. The contents of the flasks were checked visually before the next addition of solvent. For solvents which did not dissolve the test item after addition of 10 mL, the main test was not undertaken.

Determination of Solubility

The quantity of test item to be used for the solubility, generated in the preliminary test, can be derived from the following table:

Preliminary estimate of solubility (g/L)	>200	100-200	50-100	<50
Mass of test item used (g)	0.5	0.4	0.2	0.1

The test item was weighed into a volumetric flask (10 mL) and incremental measured volumes of solvent were added as indicated below, until complete dissolution.

Total volume required for complete dissolution (mL)	Solubility (g/L) where the mass of test item used is			
	0.5 g	0.4 g	0.2 g	0.1 g
2.0	>250	>200	>100	>50
2.5	200-250	160-200	80-100	40-50
3.0	167-200	133-160	67-80	33-40
3.5	/	114-133	57-67	29-33
4.0	/	100-114	50-57	25-29
5.0	/	80-100	40-50	20-25
7.0	/	/	29-40	14-20
10.0	/	/	/	10-14

After each addition of solvent, the mixture was shaken vigorously and then stored at the test temperature with intermittent shaking for 30 min. The contents of the flasks were checked visually before the next addition of solvent.

RESULTS

The solubility was determined by adding successive measured volumes of solvents to a known mass of test substance until complete dissolution. The solvents used were:

- Heptane
- Xylene
- *1,2*-Dichloroethane
- Methanol
- Acetone
- Ethyl acetate

The test was carried out at 20°(±1)°C.

The solubility in organic solvents of the test item is detailed in the following table 1 (preliminary test). As the solubility test resulted < 10 g/L in the preliminary test, the main test was not undertaken and no further investigation was done.

This method is not suitable for the determination of solubilities < 10 g/L.

Table 1: Preliminary test

Solvent	Weight (g)	Total volume required for complete dissolution (mL)							Approximate Solubility
		0.5	1.0	2.0	3.0	4.0	5.0	10.0	(g/L)
Heptane	0.1128	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	**<10**
Xylene	0.1060	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	**<10**
1,2 Dichloroethane	0.1002	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	**<10**
Methanol	0.1159	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	**<10**
Acetone	0.1058	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	**<10**
Ethyl acetate	0.1108	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	Insol.	**<10**

EXPERIMENTAL REPORT - IR ABSORPTION SPECTRUM

TEST METHOD
IR.

EXPERIMENTAL DESIGN
Organic molecules are in a constant state of vibrations, each bond having its characteristic stretching and bending frequencies. When infrared light radiations between 4000-500 cm^{-1} are passed through a sample of an organic compound, some of these radiations are absorbed by the sample and are converted into energy of a molecular vibrations. The plot of % transmittance against frequency is called the infrared spectrum of the sample or compound.

EXPERIMENTAL CONDITIONS
The IR absorption spectrum was performed using IR spectroscopy with ATR (Attenuated Total Reflectance).
The scan range was 4000-500 cm^{-1}.

REAGENTS
- Ethanol (VWR, code 20823.293, lot 18K284017)
- Mineral oil (Nujol) (Sigma Aldrich, code M3516, lot MKB57478V)

EQUIPMENT
- FT-IR Spectrometer Two FT-IR (Perkin Elmer, S/N 112253) (B1945)
- Rotavapor (Buchi), S/N 10000113229 (B1171)
- Standard laboratory equipment

The instrument used is qualified every year according internal procedure.

ANALYSIS
A small aliquot of the sample was dried by water evaporation under reduced pressure with EtOH to obtain a fine powder.
As the analysis of the powder, as itself or dispersed in a mineral oil liquid (nujol), in two Mid-Infrared transparent windows of NaCl resulted in bad quality spectra, the sample was analysed with infrared spectroscopy using ATR (Attenuated Total Reflectance) technique.

RESULTS

Considering the characteristic IR absorption frequencies, the IR spectrum of the test item has been analysed and it has been hypothesized a possible origin only for the most relevant peak, taking into account the complexity of the sample (aminoacids and peptide mixture).

Figure 1: IR absorption spectrum of the test item

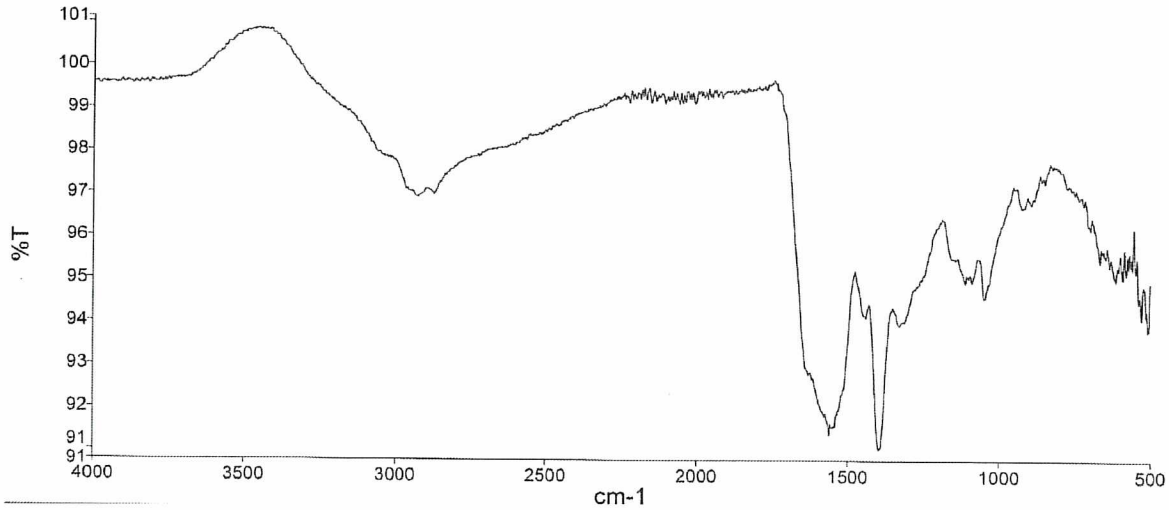

Table 2: Results on the test item

Characteristic absorptions (cm^{-1})	Functional group
≈ 3000	C-H Stetching
≈ 3000	O-H Stretching - broad signal
≈ 1500	C=O Stretching

DEVIATION

During the study a deviation has been recorded.
N.: EXC-LV19AA2492: the sample matrix showed to be not suitable for IR spectroscopy analysis with NaCl. IR spectroscopy with ATR was adopted.

CONCLUSIONS

On the basis of results obtained, the test item showed solubilities < 10 g/L in each solvent employed.
This method is not suitable for the determination of solubilities < 10 g/L.
The identity of the test item was verified performing infrared spectroscopy (IR).

ANNEXES

ANNEX	TITLE	NUMBER OF PAGES
1	Test item CoA	1

Annex #1 – Test item CoA

Soggetto a direzione e coordinamento da parte di INTESA HOLDING S.p.A. iscritta al Reg. Imprese di VI al n° 01905080244

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CERTIFICATO DI ANALISI N. 19002638

Prodotto:	HYDROLYSED PROTEINS
Codice:	110568 DO.LA.11
Lotto:	19070125
Data di produzione:	18/02/2019
Data di scadenza:	18/02/2024
Destinazione:	EUROFINS BIOLAB S.r.l.
Data di spedizione:	18/03/2019
Confezionamento:	campione per analisi di laboratorio

PARAMETRI	RISULTATI ANALITICI VALORI	UNITA' DI MISURA	METODO	SPECIFICHE	
Aspetto	Conforme			liquido bruno	
Solubilità	>1000	g/l	EPA830-7640		
Sostanza Secca	59,4	% p/p	TGA01	≥ 58,0	
Densità	1,27	g/ml	DEN01		
Azoto totale	7,60	% p/p	CNLECO01		
Azoto ammoniacale	0,55	% p/p	N03		
Azoto organico	7,05	% p/p	calcolo	≥ 6,50	
Carbonio totale	24,4	% p/p	CNLECO01	≥ 20,0	
Amminoacidi liberi	9,23	% p/p	HP02		
Amminoacidi totali	44,1	% p/p	calcolo		
Ceneri	10,6	% p/p	TGA01		
Calcio	0,24	% p/p	HP04		
Sodio	4,07	% p/p	HP04		
Cloruri	3,92	% p/p	HP04D		
Solfati	1,08	% p/p	HP04D		
pH sol. al 10%	6,40		PH01	≥ 6,00	≤ 7,00

Note:

APPROVATO Data 15/03/2019

Responsabile Controllo Qualità
Eliana Franco

15/03/2019

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