Protocol No: 3671
Date: 27/8/2013

To: Phytofil – N. Stavrakis
Cc: Dr N. Stavrakis

Laboratory methods for the evaluation of ENTOMELA 50SL

Dear Dr. Stavrakis,

Concerning the laboratory control of ENTOMELA 50SL, we would like to inform you about the methods that were used.

We determined total nitrogen, protein equivalent, ureic nitrogen, urea equivalent, nitrogen ammoniacal (expressed as N and NH_4Cl), chloride, aminoacid index, dry matter, insoluble matter in water, pH, density, color, odor, appearance. The methods used:

- AOAC 2001.11, for the total nitrogen and equivalent protein determination
- EN 15475:2009 in house method, for the ammoniacal nitrogen determination
- AOAC 959.03, for the urea nitrogen determination
- ISO 457:1983 in house method, for the chlorides determination
- AOAC 965.31 modified, for the amino acids index determination
- ISO 2920:2004 in house, for the dry matter determination
- CIPAC MT 10.2 modified, for the insoluble matter in water determination
- CIPAC MT 75.3 for the pH determination
- CIPAC MT 3.3.2 for the density determination
- Macroscopic contol for the color, the odor and the appearance determination

Briefly;

The total nitrogen determination according to AOAC 2001.11 method is based on Kjeldahl method. Nitrogen (all forms) derived from the product, is oxidized to ammonium nitrogen using catalyst (copper sulfate – potassium sulfate), sulfuric acid and high temperature for the reaction. After the digestion, a distillation is occurred. Ammonia is liberated using alkaline medium (NaOH sln) and it is collected to a standard acid medium. The excess acid is titrated by means of a standard alkaline solution. Equivalent protein is determined by multiplying total nitrogen with a factor 6,25 as method refers.

The ammoniacal nitrogen determination according to EN 15475:2009 in house method, is based on ammonia liberating after the addition of sodium hydroxide solution and the no thermal distillation of ammonia in an acid solution. The samples tested are free from proteins that previously precipitated with trichloroacetic acid.

The urea nitrogen determination according to AOAC 959.03 is based on urea hydrolysis by urease and the determination of ammonia liberated in a slightly acid environment

according to Kjeldhahl method. The samples tested are free from proteins that previously precipitated with trichloroacetic acid. Results are corrected from free ammoniacal nitrogen of samples that is determined by EN 15475 method.

The amino acids index determination according to AOAC 965.31 modified, is based on the potentiometric titration up to pH 9 after the addition of neutralized formol solution (titrated potentiometrically up to pH 9) in a sample solution neutralized (titrated potentiometrically up to pH 9).

The chloride content determination according to ISO 457:1983 by argentimetric titration, is based on precipitation of chlorides with the addition of a silver nitrate standard solution and the back titration of dilutes silver nitrate with ammonium thiocanate standard solution.

The dry matter determination according to ISO 2920:2004 is based on the determination of loss of water content after the addition of sand (previously dried) and the sample drying in an oven at 105^0C.

The insoluble matter in water is determined by CIPAC MT 10.2 method, based on cold water dissolution of sample and the filtration and weighting of insoluble matter.

Samples pH is determined by CIPAC MT 75.3 method, at temperature of 25^0C (without any dilution).

The density is determined by CIPAC MT 3.3.2 method at temperature of 20^0C (gravi-metrically, using density bottles).

Mr. Nikiforos Iliopoulos
Chemist -Environmentalist M.Sc.
HELLASCHEM Manager

ΗΛΙΟΠΟΥΛΟΣ ΝΙΚΗΦΟΡΟΣ
HELLASCHEM
ΧΗΜΙΚΕΣ ΔΟΚΙΜΕΣ ΚΑΙ ΑΝΑΛΥΣΕΙΣ
ΥΠΗΡΕΣΙΕΣ ΕΡΕΥΝΑΣ - ΕΚΠΑΙΔΕΥΣΗ
ΥΠΗΡΕΣΙΕΣ ΓΙΑ ΕΛΕΓΧΟ ΠΟΙΟΤΗΤΑΣ
ΑΦΜ: 026987995 - ΔΟΥ: ΑΜΑΡΟΥΣΙΟΥ
ΤΗΛ/ΦΑΞ: 210 6100733 info@hellaschem.gr