NUTREL/SIC

DOCUMENT M-CP, Section 5

ANALYTICAL METHODS

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[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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CP 5 ANALYTICAL METHODS

CP 5.1 Methods for the Generation of Pre-Authorisation Data

CP 5.1.1 Analysis of the Plant Protection Product

(a) Methods for the determination of the active substance and/or variant in the plant protection product

Principle: The determination of organic nitrogen is made subtracting the ammonium nitrogen content to total nitrogen content.

The method of determination of total nitrogen provides for the sample digestion with sulphuric acid at high temperatures using copper sulfate as catalyst to convert organic nitrogen to ammonia, which is distilled after alkalinization into a boric acid solution. The concentration is determined by acid-base titration(nitrogen determination according to Kjeldahl method).

The method of ammonium nitrogen determination provides for the ammonia distillation from a solution buffered at pH=7,4 into a boric acid solution. The concentration is determined by acid-base titration.

Apparatus:

Digestion unit for Kjeldahl method.

Distillation unit for Kjeldahl method.

Kjeldahl flasks.

DETERMINATION OF TOTAL NITROGEN:

Procedure :

A weighed sample (0,5g) is put in a Kjeldahl digestion flask, 20 mL of phosphosulfuric acid, the tablet containing potassium and copper sulphate are added. The sample is digested until solution clears (2 hours) and cooled.

50 mL water and 50 mL sodium hydroxide 32% w/w are added and the ammonia is distilled in 100 ml of receiving solution until neutrality.

The ammonia is titrated with standard sulphuric acid solution 0,1N until the colour change of indicator.

Calculation relative to total nitrogen content:

Total Nitrogen (% w/w)=((a-b)xNx1,4)/m

where:

a=ml mL standard sulphuric acid used to titrate the sample

b=ml mL standard sulphuric acid used to titrate the blank

N=normality relative to the sulphuric acid

m=quantity of sample (g)

DETERMINATION OF AMMONIUM NITROGEN

Procedure :

A weighed sample (1,5g) are put in a Kjeldahl digestion flask, 50 mL of buffered solution are added and the ammonia is distilled in 100 ml of receiving solution until neutrality.

The ammonia is titrated with standard sulphuric acid solution 0,1N until the colour change of indicator.

Calculation relative to total nitrogen content:

Ammonium Nitrogen (% w/w)=((a-b)xNx1,4)/m

where:

a=ml mL standard sulphuric acid used to titrate the sample

b=ml mL standard sulphuric acid used to titrate the blank

N=normality relative to the sulphuric acid

m=quantity of sample (g)

(b) Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

(c) Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

DETERMINATION OF SACCHARIDES

Principle: for the determination of the saccharides the method MP-1114 rev. 0, has been utilised.

For each sample prepare a solution weighing a quantity of sample equivalent to m, add a known quantity of internal reference material (arabinose) and fill to the mark with water. Analyse the obtained solution through HPLC equipped with electrochemical detector. Three solutions with different concentration have been prepared for each reference substance in order to trace the calibration curve used subsequently to obtain the content of each saccharide in the sample and the total content of the saccharides.

Apparatus:

- Analytical balance, precision 0,0001 g
- Chromatographic system in liquid phase and with high resolution (HPLC) composed of a gradient pump, electrochemical detector pulsated with the golden measure electrode, injector with variable volume or interchangeable loop, acquisition system and data elaboration
- 250 mm x 4 mm chromatographic column with anionic exchange filled with polymeric support (ethylvinylbenzene-divinyebenzene), diameter 8 □m agglomerated with a latex that functions with quaternary amines
- syringe filters, porosity 0,45 □m
- liquid chromatography syringe
- chromatography vials
- system for the degassing of the mobile phase
- equipment and glassware usually used in chemical laboratories

Procedure

Marking of the calibration curves: prepare solutions derived from three levels of concentration by diluting at right volumes the sugar concentrated solutions under examination. The measure of the volumes extracted from the concentrated solutions has been made with calibrated pipettes.

The data of the reference solutions used to mark the calibration curves and the analytical data obtained from the chromatographic analysis are represented in table 15.

Hereafter, the data of the calibration curves of the reference materials and the statistic parameters that define them are represented in the graphs. The acceptability of the calibration curves has been estimated in accordance with the prescription of the IDL-11-SAQ rev.0.

In a 100 ml flask weigh on an analytical balance (± 0,0001 g) about 2 g of sample, with the addition of 5 ml of the internal reference solution (arabinose) and dissolved with about 40 ml of hot deionised water (about 60º C).

Fill to the mark with demineralised water. Transfer quantitatively 5 ml, measured with a glass pipette with double mark, in a 500 ml calibrated flask and fill to the mark with deionised water. Filter the sample solution (eliminating the first 2 ml) with a syringe filter; collect in an auto sampler vial the subsequent volumes of the limpid solution and inject them in HPLC.

Calculation and expression of the results: the identification of the sugars takes place in the comparison between the retention times of the solution peaks and those of the reference solution.

The concentration w1 of each sugar, expressed in g/1000 g, has been calculated through the comparison between the area of the peaks of the sample solution normalized using the internal standard and that of the relative peaks of the reference solution. The equation is the following:

where:

area of the i-esimal sugar peak of the sample

area of the internal reference peak of the reference solution

volume of the internal reference solution added to the sample (5 ml)

mass of the i-esimal sugar in the reference solution (g)

peak area of the internal reference material of the sample

area peak of the i esimal sugar of the reference solution

volume of the internal reference solution added to the reference solution (5 ml)

sample mass

conversion factor from g/g to g/100 g

CP 5.1.2 Methods for the Determination of Residues

Hydrolysed proteins is a naturally occurring as low toxic active substance, and no MRLs are set in plants. Thus, no analytical methods are required in plants, soil, water and air, according to guideline SANCO 825/00 rev. 8.1.
Hydrolysed protein is used also as a foliar fertiliser and does not cause negative transformations in the environment, if it is used following the suggested conditions. The product is constituted by natural substances and consequently is completely biodegradable. Reasonably there are not negative effects on the environment.

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

Not required. See explanation above

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

Not required. See explanation above

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

Not required. See explanation above

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

Not required. See explanation above

(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 required. See explanation above

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

Not required. See explanation above

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

Not required. See explanation above

CP 5.2 Methods for Post-Authorisation Control and Monitoring Purposes

Hydrolysed proteins is a naturally occurring as low toxic active substance, and no MRLs are set in plants. Thus, no analytical methods are required in plants, soil, water and air, according to guideline SANCO 825/00 rev. 8.1.the hydrolysed protein is obtained by hydrolysis of animal tissues.

Hydrolysed protein from the requirement of residue data based upon the consideration that hydrolysed protein is used also as a foliar fertiliser and does not cause negative transformations in the environment, if it is used following the suggested conditions. The product is constituted by natural substances and consequently is completely biodegradable. Reasonably there are not negative effects on the environment.

Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

Not required

Methods for the determination of residues in body fluids and tissues

Not required

Methods for the determination of residues in soil

Not required

Methods for the determination of residues in water

Not required

Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Hydrolysed proteins per se are likely to be of low toxicological concern provided hydrolysed proteins of animal origin are pathogen-free and hydrolysed proteins from plant origin do not have sensitisation potential.
No risks to human health could be expected from its use as a plant protection product and data waivers for specific toxicological studies were initially supported. no risk is anticipated for the worker entering the application area directly after treatment when the same personal protective equipment with the operator is used.