SVMA14-004

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

It should be noted that grey script reflects studies already submitted and evaluated by Greece in 2017 in the context of the zonal registration of the product SVMA14-004.
The study No. 15-919069-004 was considered as not acceptable because the method quantifies total nitrogen by titration following the Kjeldahl method and applies the ratio F = 269/47 to convert it to hydrolysed proteins. The ratio F = 269/47 was based on the Certificate of Analysis of the reference active substance provided for the study.

The summary is provided below for information.

Reference:	KCP 5.5.1-01
Report	Validation of the analytical method for the determination of hydrolysed proteins in SVMA14-004 Ricau Hélène, 2015, Report No. 15-919069-004
Guideline(s):	Yes (SANCO/3030/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	No

Materials and methods

Hydrolysed proteins were analysed after extraction from the formulation and quantified by titration following the Kjeldahl method.

Preparation of the test item solutions:
A quantity of 0.3 g (to the nearest 0.01 mg) of the test item was weighed into a 250-mL round bottom flask.

Digestion step: A volume of 20 mL of sulfuric acid was slowly added. Then 1 tab of Kjeldahl catalyst and some glass balls were added. The round bottom flask was heated onto a burner under a ventilated fume cupboard for at least one and a half hour until the solution changes from brown to colourless (be careful of acid fumes). The solution was left to cool at ambient temperature and a volume of 50 mL of demineralised water was slowly added. The round bottom flask was placed in a cold water bath and a volume of 60 mL of the sodium hydroxide solution at 32% w/w was added.

Distillation step: The content of the round bottom flask was distilled and the ammonium emission was recovered in a beaker containing 100 mL of the boric acid solution at 0.1M and some drops of the methyl red solution at 0.1% w/v. At the beginning the solution was red, then became yellow with the ammonium emission.

Titration step: The nitrogen contained in the recovering beaker was titrated with the hydrochloric acid solution at 0.1M. The yellow solution became red and the end point was determined when the red colour persists more than 5 minutes.

Expression of the results:

The content of nitrogen in the test item in % w/w is:

$$C1 = \frac{V\ x\ N\ x\ M1}{Wtest\ x\ 10}$$

Where
V is the volume in mL of the hydrochloric acid solution necessary for the titration
N is the normality of the hydrochloric acid solution
M1 is the molecular mass in g/mol of nitrogen (M=14.01 g/mol)
Wtest is the weight in g of the test item

The content of hydrolysed proteins in test item in % w/w is:

$$Q = C1\ x\ F$$

Where
C1 is the content in % w/w of nitrogen in hydrolysed proteins
F is the ration between the concentration of hydrolysed proteins and nitrogen = 269/47

Validation - Results and discussions

Table 5.1-1: Methods suitable for the determination of hydrolysed proteins in plant protection product SVMA14-004

	Hydrolysed proteins
Author(s), year	Ricau Hélène, 2015
Principle of method	titration following the Kjeldahl method.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The relation between the quantity of hydrolysed proteins and the equivalent volume of hydrochloric acid was linear within the range of 40.56 mg to 120.00 mg of hydrolysed proteins, which is equivalent to 314.71 mg/L (52.8% of the declared content) to 926.33 mg/L (155.3% of the declared content). r=0.9999
Precision – Repeatability Mean n = 5 (%RSD)	The concentration of hydrolysed proteins in the test item was equal to 31.4% w/v or 314 g/L. In the case of hydrolysed proteins, the precision was acceptable as the R.S.D. was lower than the results of the modified Horwitz equation: 0.31 < 1.63 (C = 0.271)
Accuracy n = 2 (% Recovery)	The accuracy results of hydrolysed proteins were in conformity with the Guidelines requirements for formulations containing more than 10% of an active substance. Indeed, the recovery results should be in the range 98% - 102% and they were experimentally equal to 99% and 100%.
Interference/ Specificity	For the solvent blank and the formulation blank, the solution remained still red before the titration, so it was impossible to titrate with the hydrochloric acid solution at 0.1M For the reference item and the test item, nearly the same volume of the hydrochloric acid solution at 0.1M was necessary to reach the end point of the titration. The specificity is therefore defined

	Hydrolysed proteins
Comment	-

Conclusion

According to the SANCO/3030/99 rev.4 guidance document, the analytical method for the determination of hydrolysed proteins in the test item SVMA14-004 is validated.

Since the above study was considered not acceptable by the zMRS (Greece), a new analytical method and its validation No. 16-919069-002 has been performed and is provided below.

Reference:	KCP 5.5.1-02
Report	Validation of the determination of hydrolysed proteins through the validation of the analytical methods for the determination of total nitrogen and of ammonium nitrogen in SVMA14-004, Ricau Hélène, 2017, Report No. 16-919069-002
Guideline(s):	Yes (SANCO/3030/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the test is to validate the determination of proteins hydrolysed through the validation of the analytical methods for the determination of total nitrogen and ammonium nitrogen in SVMA14-004 following SANCO/3030/99 rev. 4 from 11/07/00.

Total nitrogen is analysed after extraction from the formulation and quantified by titration following the Kjeldahl method (part of determination of total nitrogen).

Ammonium nitrogen is analysed after extraction from the formulation and quantified by titration following the Kjeldahl method (part of determination of ammonium nitrogen).

- *Part of determination of total nitrogen*

Preparation of the test item solutions:
A quantity of 0.3 g of the test item was weighed (to the nearest 0.01 mg) into a 250-mL round bottom flask.

Digestion step: A volume of 20 mL of sulfuric acid was slowly added. Then 1 tab of Kjeldahl catalyst and some glass balls were added. The round bottom flask was heated onto a burner under a ventilated fume cupboard until the solution changes from brown to colourless (be careful of acid fumes). The solution was left to cool at ambient temperature and a volume of 50 mL of demineralised water was slowly added. The round bottom flask was placed in a cold water bath and a volume of 60 mL of the sodium hydroxide solution at 32% w/w was added.

Distillation step: The content of the round bottom flask was distilled and the ammonium emission was recovered in a beaker containing 100 mL of the boric acid solution at 0.1M and some drops of the methyl red solution at 0.1% w/v. At the beginning the solution was red, then became yellow with the ammonium emission.

Titration step: The nitrogen contained in the recovering beaker was titrated with the hydrochloric

acid solution at 0.1M. The yellow solution became red and the end point was determined when the red colour persists more than 5 minutes.

Expression of the results:
The content of nitrogen in the test item in % w/w is:

$$Q = \frac{V \; x \; N \; x \; M \; x \; d}{Wtest \; x \; 10}$$

Where
V is the volume in mL of the hydrochloric acid solution necessary for the titration
N is the normality of the hydrochloric acid solution
M is the molecular mass in g/mol of nitrogen (M=14.01 g/mol)
Wtest is the weight in g of the test item
D is the density in g/mL of the test item (=1.16 from analytical certificate)

- *Part of determination of ammonium nitrogen*

Preparation of the test item solutions:
A quantity of 3 g of the test item was weighed (to the nearest 0.01 mg) into a 250-mL round bottom flask. A volume of 50 mL of the sodium hydroxide solution at 0.1M was added and few small glass balls were added.

Distillation step: The content of the round bottom flask was distilled and the ammonium emission was recovered in a beaker containing 100 mL of the boric acid solution at 0.1M and some drops of the methyl red solution at 0.1% w/v. At the beginning the solution was red, then became yellow with the ammonium emission.

Titration step: The nitrogen contained in the recovering beaker was titrated with the hydrochloric acid solution at 0.1M. The yellow solution became red and the end point was determined when the red colour persists more than 5 minutes.

Expression of the results:
The content of nitrogen in the test item in % w/w is:

$$Q = \frac{V \; x \; N \; x \; M \; x \; d}{Wtest \; x \; 10}$$

Where
V is the volume in mL of the hydrochloric acid solution necessary for the titration
N is the normality of the hydrochloric acid solution
M is the molecular mass in g/mol of nitrogen (M=14.01 g/mol)
Wtest is the weight in g of the test item
D is the density in g/mL of the test item (=1.16 from analytical certificate)

Validation - Results and discussions

Table 5.1-2: Methods suitable for the determination of hydrolysed proteins in plant protection product SVMA14-004

	Hydrolysed proteins
Author(s), year	Ricau Hélène, 2017
Principle of method	Titration following the Kjeldahl method
Part of determination of total nitrogen	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The relation between the quantity of total nitrogen and the equivalent volume of hydrochloric acid was linear within the range of 56.20 mg/L to 162.40 mg/L (7.35 mg to 20.99 mg) of total nitrogen. $y= 7.25.10^{-1} x – 2.34.10^{-1}$ r=0.9997
Precision – Repeatability Mean n = 5 (%RSD)	The concentration of total nitrogen in the test item was equal to 5.52% w/v or 55.2 g/L. In the case of total nitrogen, the precision was acceptable as the R.S.D. was lower than the results of the modified Horwitz equation: 0.99 < 2.12 (C = 0.048)
Accuracy n = 2 (% Recovery)	The accuracy results of total nitrogen were in conformity with the Guidelines requirements for formulations containing between 1 and 10% of an active substance. Indeed, the recovery results should be in the range 97% - 103% and they were experimentally equal to 98.7% and 98.9%.
Interference/ Specificity	For the solvent blank and the formulation blank, a volume of 0.1 mL of the hydrochloric acid solution at 0.1M was observed to reach the end point of the titration. As this volume is less than 3% of the volume necessary to reach the end point of the titration in the test item, there was no impact on the calculation. For the reference item and the test item, nearly the same volume (10.0 mL and 10.5mL, respectively) of the hydrochloric acid solution at 0.1M was necessary to reach the end point of the titration. The specificity is for total nitrogen therefore defined
Comment	-
Part of determination of ammonium nitrogen	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The relation between the quantity of ammonium nitrogen and the equivalent volume of hydrochloric acid was linear within the range of 124.54 mg/L to 370.71 mg/L (6.32 mg to 18.67 mg) of ammonium nitrogen. $y= 6.72 \times 10^{-1} x + 4.57\ 10^{-1}$
Precision – Repeatability Mean n = 5 (%RSD)	The concentration of ammonium nitrogen in the test item was equal to 0.49% w/v or 4.9 g/L. In the case of ammonium nitrogen, the precision was acceptable as the R.S.D. was lower than the results of the modified Horwitz equation: 0.77 < 3.05 (C = 0.0042)
Accuracy n = 2 (% Recovery)	The accuracy results of ammonium nitrogen were in conformity with the Guidelines requirements for formulations containing between 0.1% and 1% of an active substance. Indeed, the recovery results should be in the range 95% - 105% and they

	Hydrolysed proteins
	were experimentally equal to 100% and 101.2%.
Interference/ Specificity	For the solvent blank and the formulation blank, a volume of 0.1 mL of the hydrochloric acid solution at 0.1M was observed to reach the end point of the titration. As this volume is less than 3% of the volume necessary to reach the end point of the titration in the test item, there was no impact on the calculation. For the reference item and the test item, nearly the same volume (8.7 mL and 9.0mL, respectively) of the hydrochloric acid solution at 0.1M was necessary to reach the end point of the titration. The specificity for ammonium nitrogen is therefore defined
Comment	-

❖ *Content of organic nitrogen*

The content of organic nitrogen was calculated by the difference between the content of total nitrogen and the content of ammonium nitrogen. The content of organic nitrogen found was 5.03% w/v.

❖ *Content expressed in hydrolysed proteins*

The content of hydrolysed proteins in test item is expressed by multiplying the organic nitrogen content by the conventional factor of 6.25. The content of hydrolysed proteins found was 31.4 % w/v.

Conclusion

According to the SANCO/3030/99 rev.4 guidance document, the analytical method for the determination of hydrolysed proteins in the test item SVMA14-004 is validated.

(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

No method was developed for the determination of relevant impurities since the product SVMA14-004 does not contain relevant impurities of toxicological, ecotoxicological or environmental significance (either formed during manufacture or from degradation). Please refer to Document J.

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

With respect to toxicological, ecotoxicological or environmental aspects, the product does not contain any relevant co-formulants. Therefore, a specific analytical method and validation is not required. Please refer to Document J.

CP 5.1.2 Methods for the Determination of Residues

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

There are no methods required. Please refer to Document M-CA 4.

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

There are no methods required. Please refer to Document M-CA 4.

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

There are no methods required. Please refer to Document M-CA 4.

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

There are no methods required. Please refer to Document M-CA 4.

(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

There are no methods required. Please refer to Document M-CA 4.

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

There are no methods required. Please refer to Document M-CA 4.

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

There are no methods required. Please refer to Document M-CA 4.

CP 5.2 Methods for Post-Authorisation Control and Monitoring Purposes

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

There are no methods required. Please refer to Document M-CA 4.

Methods for the determination of residues in body fluids and tissues

There are no methods required. Please refer to Document M-CA 4.

Methods for the determination of residues in soil

There are no methods required. Please refer to Document M-CA 4.

Methods for the determination of residues in water

There are no methods required. Please refer to Document M-CA 4.

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

There are no methods required. Please refer to Document M-CA 4.