FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 123000 A

Product name: AZA

Chemical active substance:

Azadirachtin, 10 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: SHARDA Cropchem España S.L.

Submission date: October 2020

Update date: May 2021

Finalisation date: 07/2021

Version history

When	What
July 2020	Submission
May 2021	Updated by applicant
July 2021	ZRMs evaluated dRR submitted by Applicant.

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance and relevant impurities in the plant protection product.
Noticed data gaps are:

- None
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Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

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- Noticed data gaps are:
- Appendix 1, Table List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review should be completed (minor deficiencies)
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• **Commodity/crop**	• **Supported/ Not supported**
• Tomato	• Supported
• Potato	• Supported
• Ornamentals	• Not required

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of Azadirachtin in plant protection product is provided as follows:

Comments of zRMS:	The analytical method meets the criteria of specificity, linearity, precision and accuracy. The method is acceptable and is suitable for determination of azadirachtrin in plant protection product Aza

Azadirachtin A content in formulation SHA123000A is determined by standard official method CIPAC 627/TK/M/3 (CIPAC Handbook M, 2009), by reverse phase HPLC and UV detection at 214 nm with external standard.

~~Reference standard is prepared in Methanol/water. EC formulation sample is prepared also in Metha-~~

~~nol/water and sonication.~~

~~Chromatographic parameters:~~
- ~~Column Luna 150x4.6 mm, 3 µm or similar.~~
- ~~Mobile phase: acetonitrile/water (44/56 v/v).~~
- ~~Column temperature: 30ºC.~~
- ~~Flow rate: 1.1 ml/min~~
- ~~Detection: 214 nm.~~

~~Conclusion~~

~~Official CIPAC standard method is available.~~

Reference:	KCP 5.1.1
Report	Accelerated storage stability test by heating at elevated temperature of Azadirachtin 1% EC. D. Bangera, 2020, Report No. G12479
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for the determination of active ingredient (a.i.) content in the test item was validated by establishing specifity, Linearity, Range, Limit of Detection (LOD), Limit of Qunatification (LOQ), Precision and Accuracy.

Linearity of Detector Response and Range
About 0.02411 g of Azadirachtin reference standard was accurately weighed into a 50 mL volumetric flask and the contents of the flask were dissolved in about 20 mL of methanol and sonicated for 3 minutes. After equilibrating to room temperature solution was made up to the mark with methanol. Shaken thoroughly for homogeneity.
For detector linearity check, five working standard solutions of different concentrations of the active ingredient were prepared from the stock solution of Azadirachtin reference standard with methanol and injected in triplicate to HPLC.

Specifity
The specifity of the method for active ingredient was studied by injecting individual solutions of workins standard solution, test item solution and analytical blank (Acetonitrile) to the HPLC operated under the same chromatographic conditions. The interferences at the retention time of active ingredient in blank injection were studied.

Precision
The Azadirachtin concentration without purity correction was 78.12 µg/mL. This solution was used as working standard for precision test.
About 0.5 g of the test item, in five replications was taken into separate 100 mL volumetric flasks, dissolved in about 20 mL of Acetonitrile and sonicated for 2 minutes. After equilibrating to room temperature, these solutions were made up to the mark with the acetonitrile and shaken well for homogeneity. These solutions were then analyzed for the active ingredient content by injecting to HPLC under the same chromatographic conditions.

Accuracy
From the stock solution of Azadirachtin reference standard (concentration – 482 µg/mL), DLC-3 solution without purity correction used as working standard (Concentration 78.12 µg/mL)
About 0.05 g test item was weighed in triplicate at each of three fortification levels (90, 100 and 110% of nominal concentration in test item) into 10 mL volumetric flasks. To these volumetric flasks, 1.1 mL, 1.15, and 1.3 mL of stock solution of Azadirachtin reference standard (prepared during detector linearity check with concentration 402µg/mL) was added. Dissolved the contents in of acetonitrile and sonicated for 2 minutes. After equilibrating to room temperature, the volume was made up to the mark with the same. Shaken well for homogeneity. These fortified samples were analyzed for active ingredient content using HPLC.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances Azadirachtin in plant protection product AZA (SHA 123000 A)

	Azadirachtin
Author(s), year	D. Bagnera, 2020
Principle of method	HPLC
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	5 points 37.42 to 121.61 µg/mL R = 1.000 y=6549.573x-8472.877
Precision – Repeatability Mean n = 5 (%RSD)	%RSD = 0.88% Horrat value = 0.34
Accuracy n = 3 (% Recovery)	90%: 99.51 ± 0.25 100%: 97.54 ± 0.67 110%: 101.03 ± 0.34%
Interference/ Specificity	No interference. The method is specific.
Comment	

Conclusion

According to SANCO/3030/99 rev. 5 the method was successfully validated and is suitable for determination of Azadirachtin content in the test item Azadirachtin 1% EC.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	The analytical method meets the criteria of specificity, linearity, precision and accuracy. The method is acceptable and is suitable for determination of relevant impurities Aflatoxin-G2, Aflatoxin – G1, Aflatoxin – B2 and Aflatoxin – B1 in plant protection product Aza.

~~No method was submitted for the determination of aflatoxins in the preparations. However, it seems that such a method is not required since it is unlikely that aflatoxins will be formed during the formulation process or storage.~~

Reference:	KCP 5.1.1-2
Report	Accelerated storage stability test by heating at elevated temperature of Azadirachtin 1% EC. D. Bangera, 2020, Report No. G12479
Guideline(s):	SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for the determination of Aflatoxin-G2, Aflatoxin – G1, Aflatoxin – B2 and Aflatoxin – B1 contents in test item was validated by establishing specifity and selectivity, linearity, range, LOD and LOQ, system precision, method precision, intermediate precision and accuracy.

Specifity and Seletivity
The specifity and selectivity of the method was established by injecting diluent along with the Aflatoxins working standard solutions to the LC-MS/MS.

Linearity of Detector Response and Range
Different aliquots from the Aflatoxins reference standard stock solutions prepared was transferred into a volumetric flask and diluted with diluent to get five different working standard solutions for detector linearity check.

Precision
About 0.8 g of the test item, Azadirachtin 1% EC formulation was weighed in five replications to separate 5 mL volumetric flasks and the contents of the flask were dissolved in methanol by sonicating for 3 minutes. After equilibrating to room temperature, the volume was made up to the mark with the same, the solution was shaken thoroughly. An aliquot of 5 mL of each of these solutions was transferred into 20 mL volumetric flask and diluted using Phosphatee buffered saline (PBS) and further the solution was cleaned up.

Accuracy
About 0.8 g of test item, Azadirachtin 1% EC Formulation was weighed into separate 5 mL volumetric flasks, fortified with mixed solution of 0.0096 µg/mL of Aflatoxin-G2, 0.0384 µg/mL of Aflatoxin-G2, 0.0092 µg/mL of Aflatoxin-B2, and 0.0382 µg/mL of Aflatoxin-B1 and the contents of the volumetric flasks were dissolved in methanol by sonicating for 3 minutes. After equilibrating to room temperature, the volume was made up to the matk with methanol. The solutions were shaken thoroughly. An aliquot of 5 mL of each of these solutions was transferred into 20 mL volumetric flask and diluted using Phosphated buffered saline and further the solution was cleaned up and used for the analysis of Aflatoxin-G2, Aflatoxin-G1, Aflatoxin-B2 and Aflatoxin-B1 content by injecting to LC-MS/MS.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of relevant impurities Aflatoxin-G2, Aflatoxin-G1, Aflatoxin-B2 and Aflatoxin-B1 in plant protection product AZA (SHA 123000 A)

	Aflatoxin-G2	Aflatoxin-G1	Aflatoxin – B2	Aflatoxin – B1
Author(s), year	D. Bagnera, 2020			
Principle of method	HPLC			
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	5 points 0.0000480 to 0.001920 µg/mL R = 0.996 y=204908421.2x-4080.22	5 points 0.0001920 to 0.00768 µg/mL R = 1.00 y=532302341.28x-13086.65	5 points 0.0000460 to 0.001840 µg/mL R = 0.9998 y=673567983.45x-13337.72	5 points 0.0001910 to 0.007640 µg/mL R = 1.00 y=794845007.86x-19381.19
Precision – Repeatability Mean n = 5 (%RSD)	Method precision: 0% (as analyte <LOD) System precision: 4.414%	Method precision: 0% (as analyte <LOD) System precision: 3.154%	Method precision: 0% (as analyte <LOD) System precision: 4.207%	Method precision: 0.000000037% (as analyte <LOD) System precision: 3.074%
Accuracy n = 3 (% Recovery)	Overall mean accuracy 96.473 ± 11.012	Overall mean accuracy 99.561 ± 5.970	Overall mean accuracy 104.503 ± 5.617	Overall mean accuracy 89.34 ± 11.057
Interference/ Specificity	No interference, the method is specific.			
Comment	LOD = 0.00000000053% w/w LOQ = 0.00000007 % w/w	LOD = 0.00000000031% w/w LOQ = 0.00000026 % w/w	LOD = 0.00000000022% w/w LOQ = 0.0000000506 % w/w	LOD = 0.00000000025% w/w LOQ = 0.00000026 % w/w

Conclusion

According to SANCO/3030/99 rev. 5 the method was successfully validated and is suitable for determination of relevant impurities Aflatoxin-G2, Aflatoxin-G1, Aflatoxin-B2 and Aflatoxin-B1content in the test item Azadirachtin 1% EC.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Under current EU legislation, analytical methods on formulants are not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 627 is available for Azadirachtin A.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to post-registration methods.

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product are already submitted in accordance with the requirements set out in point 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of Azadirachtin (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Azadirachtin A	1 mg/kg	Reg. (EC) 149/2008
Muscle	Not defined	0.01 mg/kg	Reg. (EC) 149/2008
Milk		0.01 mg/kg	Reg. (EC) 149/2008
Eggs		0.01 mg/kg	Reg. (EC) 149/2008
Fat		0.01 mg/kg	Reg. (EC) 149/2008
Liver, kidney		0.01 mg/kg	Reg. (EC) 149/2008
Soil (Ecotoxicology)	Not defined	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Not defined	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Not defined	1.6 µg/L	Lowest NOEC from aquatic toxicity study on *C. riparius*
Air	Not defined	30 µg/m^3	AOEL sys/AOEL inhal: 0.1 mg/kg bw/d
Tissue (meat or liver)	Not relevant	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Azadirachtin in plant matrices is given in the following tables.

Evaluator comments:
Food/feed of plant origin (*EFSA Journal 2018;16(4):5234*, analytical technique and LOQ for methods for monitoring purposes)
Azadirachtin A
LC-MS/MS; LOQ: 0.02 mg/kg (beans, cabbage, lettuce, cucumber, melon, peaches, strawberries grapes,

peppers, orange);
LC-MS/MS; LOQ: 0.02 mg/kg (cucumber, lemon balm);
HPLC-UV; LOQ: 0.01 mg/kg (potato);
HPLC-UV; LOQ: 0.01 mg/kg (tomato);
HPLC-UV; LOQ: 0.1 mg/kg (spinach);
Azadirachtin A and Azadirachtin B
HPLC-UV; LOQ: 0.02 mg/kg (apple);
Note: All HPLC-UV methods are not validated in an independent laboratory. However, the LC-MS/MS ones are validated in an independent laboratory.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: Azadirachtin A				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.02 mg/kg	LC-MS/MS	Mende, P., 2003 / EU agreed (DAR 2008)
	ILV	0.02 mg/kg	LC-MS/MS	Class, T., 2007 / EU agreed (DAR 2008)
	Confirmatory (if required)	0.02 mg/kg	LC-MS/MS	Mende, P., 2003 / EU agreed (DAR 2008)

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Not provided during the EU review

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

According to EFSA Conclusions (EFSA Journal 2018;16(4):5234), no monitoring or enforcement methods are available for food and feed of animal origin since no relevant residues are expected from the representative uses.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

Evaluator comments:
Soil *EFSA Journal 2018;16(4):5234*, analytical technique and LOQ for methods for monitoring purposes)
Azadirachtin A
LC-MS/MS 0.02 mg/kg (standard soil);
Pending on the final residue definitions, data gaps might be identified for enforcement analytical methods

An overview on the acceptable methods and possible data gaps for analysis of Azadirachtin in soil is giv-

en in the following tables.

Table 5.3-4: Validated methods for soil

Component of residue definition: Azadirachtin A			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.02 mg/kg	LC-MS/MS	Witte, A., 2008 / EU agreed (DAR 2008)
Confirmatory	0.02 mg/kg	LC-MS/MS	Witte, A., 2008 / EU agreed (DAR 2008)

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

Evaluator comments:
Water *EFSA Journal 2018;16(4):5234,* analytical technique and LOQ for methods for monitoring purposes)
Azadirachtin A
HPLC-UV 1 µg/L (surface water);
LC-MS/MS 0.05 µg/L (drinking water, surface water);
Pending on the final residue definitions, data gaps might be identified for enforcement analytical methods.

An overview on the acceptable methods and possible data gaps for analysis of Azadirachtin in surface and drinking water is given in the following tables.

Table 5.3-5: Validated methods for water

Component of residue definition: Azadirachtin A				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Class, T. 2007 / EU agreed (DAR 2008)
	ILV	-	-	-
	Confirmatory	0.05 µg/L	LC-MS/MS	Class, T. 2007 / EU agreed (DAR 2008)
Surface water	Primary	0.05 µg/L	LC-MS/MS	Class, T. 2007 / EU agreed (DAR 2008)
	Confirmatory	0.05 µg/L	LC-MS/MS	Class, T. 2007 / EU agreed (DAR 2008)

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

Evaluator comments:

Water *EFSA Journal 2018;16(4):5234,* analytical technique and LOQ for methods for monitoring purposes)
Azadirachtin A
LC-MS/MS 3 µg/m³ (ambient air, warm humid air); TRF, SIP, Mitsui
Pending on the final residue definitions, data gaps might be identified for enforcement analytical methods.

An overview on the acceptable methods and possible data gaps for analysis of Azadirachtin in air is given in the following tables.

Table 5.3-6: Validated methods for air

Component of residue definition: Azadirachtin A			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	3 µg/m^3	LC-MS/MS	Class, T. 2007 / EU agreed (DAR 2008)
Confirmatory	3 µg/m^3	LC-MS/MS	Class, T. 2007 / EU agreed (DAR 2008)

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

The active substance is not classified as toxic according to Regulation (EC) No 1272/2008 (CLP Regulation), therefore a method of analysis is not required for body fluids and tissues according to EFSA Conclusions (EFSA Journal 2018;16(4):5234).

5.3.2.8 Other studies/ information

There are no additional European requirements for formulated products..

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1 KCP 5.1.1-2	D. Bagnera	2020	Accelerated storage stability test by heating at elevated temperature of Azadirachtin 1% EC. Eurofins Advinus Limited Report No. G12479 GLP Unpublished	N	Sharda Cropchem Limited

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Azadirachtin

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted