REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB2111F

Product name(s): Starinta

Chemical active substance(s):

Bixafen, 125 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: December 2023

zRMS Assessment : 09/08/2024

Version after commenting : 15/11/2024

Version history

When	What
August 2024	zRMS assesment
November 2024	zRMS: after first round of commenting

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5 Analytical methods

5.1 Conclusion and summary of assessment

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

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

- no data gaps

Commodity/crop	Supported/ Not supported
Winter cereals	supported
Spring cereals	supported

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 bixafen in plant protection product is provided as follows:

Comments of zRMS:	The analytical method code: AG-G0105 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance bixafen. The method is successfully validated and accepted.

Reference:	KCP 5.1.1
Report	Purity analysis of GLOB2111F, Kishora K.S., 2023, AG-G0105, Eurofins Advinus Agrosciences Services India Private Limited
Guideline(s):	Yes (SANCO 3030/99 rev.5)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

The content of bixafen was determined using an in-house developed and validated High Performance Liquid Chromatographic (HPLC) analytical method.

The active ingredient content in test item was determined under the chromatographic conditions given below:

Instrument :	High Performance Liquid Chromatograph equipped with DAD and PC based data system.
Column :	Eclipse XDB-C18, 150 mm long, 4.6 mm internal diameter, 5 µm particle size.
Column Temperature:	30°C
Mobile Phase A:	0.1 % OPA in Milli-Q Water (45%)
Mobile Phase B:	Acetonitrile (55%)
Solvent Flow Rate:	1.0 mL/min.
Detector wavelength :	238 nm
Injection Volume:	10 µL
Run time:	5 minutes

All the parameters were maintained constant throughout the analysis.

Peak in the sample was identified by comparing the retention time with those obtained through the separate injection of standard solution and the absence of such peak in the control (diluent solvent) sample was checked.

Before and after sample injections, an injection of calibration (standard) solution was made, and the average peak area was used for the calculation of % active ingredient content in the test item.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of the active substance bixafen in plant protection product GLOB2111F

	Bixafen
Author(s), year	Kishora K.S., 2023
Principle of method	Transfer about 0.5 g of test item into separate 100 mL volumetric flasks, and dissolve the contents of the flask in approx. 60 mL acetonitrile by sonicating for 2 minutes. After equilibrating to room temperature, made up the volume to the mark with acetonitrile. Further, transfer an 4 mL aliquot of the stock solution into 50 mL volumetric flask and dilute to the volume with acetonitrile. Shake the solution thoroughly and used for analysis in the HPLC-DAD with the conditions specified above.
Linearity	Instrument response was linear in the range of 10.622 µg/mL to 159.329 µg/mL R^2 = 0.99868.
Precision – Repeatability Mean n = 5 (%RSD)	The precision of the method was found to be 0.79 % RSD, which was found to be within the limit i.e ≤ 1.83 % Hr = 0.43
Accuracy n = 5 (% Recovery)	The accuracy of the method ranged between 98.87 and 101.56 % with an overall mean ± s.d. of 99.9 ± 1.1 %.
Interference/ Specificity	There was no interference observed at the retention time of the analyte (a.i.) indicating the specificity of the analytical method.

	Bixafen
Comment	All validity criteria were met.

Conclusion

The analytical method was successfully validated for the determination of bixafen in GLOB2111F according to the requirements laid down by SANCO3030/99.

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

GLOB2111F does not contain any relevant impurities.

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

With respect to toxicological, eco-toxicological or environmental aspects the product GLOB2111F does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed. Insert details on the analytical methods for formulants (when required)

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of bixafen in formulations.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of bixafen for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: bixafen and desmethyl-bixafen (M21)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food/feed of plant origin (Residues)	Primary	Bixafen: 0.01 mg/kg desmethyl-bixafen: 0.01 mg/kg (expressed as bixafen) Sum of bixafen and desmethyl-bixafen expressed as bixafen: 0.02 mg/kg	LC-MS/MS	~~Bardel &~~ Schöning, 2006 EFSA, 2012 UK, 2012

	Confirmatory	-	Not required	-
Food/feed of animal origin (Residues)	Primary	Bixafen: 0.01 mg/kg desmethyl-bixafen: 0.01 mg/kg (expressed as bixafen) Sum of bixafen and desmethyl-bixafen expressed as bixafen: 0.02 mg/kg		~~Billian & Druskus, 2007~~ Schoening & Willmess, 2007 EFSA, 2012 UK, 2012
	Confirmatory	-	Not required	-
Component of residue definition: bixafen				
Soil (Environmental fate)	Primary	0.005 mg/kg	LC-MS/MS	Brumhard & Freitag, 2006 EFSA, 2012 UK, 2012
	Confirmatory	-	Not required	-
Water surface & drinking (Environmental fate)	Primary	0.05 mg/kg	LC-MS/MS	Krebber & Braune, 2008 EFSA, 2012 UK, 2012
	Confirmatory	-	Not required	-
Air (Environmental fate)	Primary	10 µg/m^3	LC-MS/MS	Class, 2007 EFSA, 2012 UK, 2012
	Confirmatory	-	Not required	-
Water (Ecotoxicology)	Primary	0.097 mg/L	HPLC-DAD	Ciorga, 2023a
AAP medium, M7 medium (Ecotoxicology)	Primary	0.097 mg/L	HPLC-DAD	Ciorga, 2023a
	Primary	0.011 mg/L	HPLC-DAD	Ciorga, 2023b
Feeding solution (Ecotoxicology)	Primary	0.1 mg/mL	HPLC-DAD	Ballai, 2023a
Surface treat solution (Ecotoxicology)	Primary	1 mg/mL	HPLC-DAD	Ballai, 2023b

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 shall be submitted, unless the applicant shows that these methods already submitted in

accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of bixafen (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	Bixafen	0.01mg/kg (LOQ)	Regulation (EU) 2023/1069 EFSA, 2012 UK, 2012
Plant, high acid content		0.01mg/kg (LOQ)	Regulation (EU) 2023/1069 EFSA, 2012
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg* - 1.5 mg/kg (cereals) 0.01mg/kg (LOQ)	UK, 2012
Plant, high oil content		0.01mg/kg (LOQ)	Regulation (EU) 2023/1069 EFSA, 2012
Plant, difficult matrices (hops, spices, tea)		0.01mg/kg (LOQ)	UK, 2012
Muscle	Sum of bixafen and desmethyl bixafen (M21), expressed as bixafen	Bixafen + desmethyl-bixafen: 0.02 mg/kg (LOQ) 0.02 mg/kg (lowest MRL poultry)	Regulation (EU) 2023/1069 EFSA, 2012
Milk		Bixafen + desmethyl-bixafen: 0.02 mg/kg (LOQ) 0.2 mg/kg (MRL)	Regulation (EU) 2023/1069 EFSA, 2012 UK, 2012
Eggs		Bixafen + desmethyl-bixafen: 0.02 mg/kg (LOQ) 0.05 mg/kg (MRL)	Regulation (EU) 2023/1069 EFSA, 2012 UK, 2012
Fat		Bixafen + desmethyl-bixafen: 0.02 mg/kg (LOQ) 0.05 mg/kg (lowest MRL poultry)	Regulation (EU) 2023/1069 EFSA, 2012 UK, 2012
Liver, kidney		Bixafen + desmethyl-bixafen: 0.02 mg/kg (LOQ) 0.05 mg/kg (lowest MRL poultry)	Regulation (EU) 2023/1069 EFSA, 2012 UK, 2012
Soil	bixafen	0.005 mg/kg	EFSA, 2012

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
(Ecotoxicology)			UK, 2012
Drinking water (Human toxicology)	bixafen	0.05 µg/L (LOQ)	EFSA, 2012 UK, 2012
Surface water (Ecotoxicology)	bixafen	0.05 µg/L (LOQ)	EFSA, 2012 UK, 2012
Air	bixafen	10 µg/m^3	EFSA, 2012 UK, 2012
Tissue (meat or liver)	Sum of bixafen and desmethyl bixafen, expressed as bixafen	Limit 0.1 mg/kg	SANCO/825/00 rev.8.1
Body fluids		Limit 0.05 mg/L	SANCO/825/00 rev.8.1

(1) Only MRL of the intended uses and the lowest MRL for each matrix type should are reported.

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 bixafen in plant matrices is given in the following tables.

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: bixafen				
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	0.01 mg/kg	LC-MS/MS, confirmation included	Bardel & Schöning, 2006 EFSA, 2012 UK, 2012
	ILV			Matrix type not included
	Confirmatory (if required)	0.01 mg/kg	HPLC-MS/MS Confirmation by 2 mass transitions in the primary method	See above
High acid content	Primary	0.01 mg/kg	LC-MS/MS, confirmation included	Bardel & Schöning, 2006 EFSA, 2012 UK, 2012
	ILV	0.01 mg/kg	LC-MS/MS	Ballesteros & Portet, 2008 EFSA, 2012 UK, 2012
	Confirmatory (if required)	0.01 mg/kg	HPLC-MS/MS Confirmation by 2 mass transitions in the primary method and ILV	See above

Component of residue definition: bixafen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High oil content	Primary	0.01 mg/kg	LC-MS/MS, confirmation included	Bardel & Schöning, 2006 EFSA, 2012 UK, 2012
	ILV	0.01 mg/kg	LC-MS/MS	Ballesteros & Portet, 2008
	Confirmatory (if required)	0.01 mg/kg	HPLC-MS/MS Confirmation by 2 mass transitions in the primary method and ILV	See above
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS, confirmation included	Bardel & Schöning, 2006 EFSA, 2012 UK, 2012
	ILV	0.01 mg/kg	LC-MS/MS	Ballesteros & Portet, 2008
	Confirmatory (if required)	0.01 mg/kg	HPLC-MS/MS Confirmation by 2 mass transitions in the primary method and ILV	See above
Difficult (if required, depends on intended use)	Not required			

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:	The method validated in Bardel & Schöning (2006) uses the same extraction methodology as the analytical method used during the metabolism study in cereals (wheat grain and forage). Further extraction efficiency testing is therefore not required.

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

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

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: sum of bixafen and desmethyl-bixafen, expressed as bixafen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.02 mg/kg	LC-MS/MS, confirmation included	Billian & Druskus, 2007 EFSA, 2012 UK, 2012
	ILV	0.02 mg/kg	LC-MS/MS	Ballesteros, 2007
	Confirmatory (if required)	0.02 mg/kg	HPLC-MS/MS Con-firmation by 2 MRM transitions in the primary method and ILV.	Se above
Eggs	Primary	0.02 mg/kg	LC-MS/MS, confirmation included	Billian & Druskus, 2007 EFSA, 2012 UK, 2012
	ILV	0.02 mg/kg	LC-MS/MS	Ballesteros, 2007
	Confirmatory (if required)	0.02 mg/kg	HPLC-MS/MS Confirmation by 2 MRM transitions in the primary method and ILV.	Se above
Muscle	Primary	0.02 mg/kg	LC-MS/MS, confirmation included	Billian & Druskus, 2007 EFSA, 2012 UK, 2012
	ILV			Matrix not included
	Confirmatory (if required)	0.02 mg/kg	HPLC-MS/MS Confirmation by 2 MRM transitions in the primary method.	Se above
Fat	Primary	0.02 mg/kg	LC-MS/MS, confirmation included	Billian & Druskus, 2007 EFSA, 2012 UK, 2012
	ILV	0.02 mg/kg	LC-MS/MS	Ballesteros, 2007 EFSA, 2012 UK, 2012
	Confirmatory (if required)	0.02 mg/kg	HPLC-MS/MS Confirmation by 2 MRM transitions in the primary method and ILV.	Se above
Kidney, liver	Primary	0.02 mg/kg	LC-MS/MS, confirmation included	Billian & Druskus, 2007 EFSA, 2012 UK, 2012

Component of residue definition: sum of bixafen and desmethyl-bixafen, expressed as bixafen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.02 mg/kg	LC-MS/MS	Ballesteros, 2007 EFSA, 2012 UK, 2012
	Confirmatory (if required)	0.02 mg/kg	HPLC-MS/MS Confirmation by 2 MRM transitions in the primary method and ILV.	Se above

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	/
Not required, because:	The method validated in Billian & Druskus (2007) uses the same extraction methodology as the analytical method used in the metabolism studies on goat and hen. Further extraction efficiency testing is therefore not required.

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

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

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: bixafen			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.005 mg/kg	LC-MS/MS	Brumhard & Freitag, 2006 EFSA, 2012 UK, 2012
Confirmatory	0.005 mg/kg	LC-MS/MS	Brumhard & Freitag, 2006 EFSA, 2012 UK, 2012

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)

An overview on the acceptable methods and possible data gaps for analysis of bixafen in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is

referred to Appendix 2.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: bixafen				
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	Krebber & Braune, 2008 EFSA, 2012 UK, 2012
	Confirmatory	0.05 µg/L	LC-MS/MS	Krebber & Braune, 2008 EFSA, 2012 UK, 2012
Surface water	Primary	0.05 µg/L	LC-MS/MS	Krebber & Braune, 2008 EFSA, 2012 UK, 2012
	Confirmatory	0.05 µg/L	LC-MS/MS	Krebber & Braune, 2008 EFSA, 2012 UK, 2012

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)

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

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: bixafen			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	10 µg/m^3	LC-MS/MS	Class, 2007 EFSA, 2012 UK, 2012
Confirmatory	10 µg/m^3	LC-MS/MS	Class, 2007 EFSA, 2012 UK, 2012

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)

An overview on the acceptable methods and possible data gaps for analysis of bixafen in body fluids and tissues is given in the following table. For the detailed evaluation of the new study it is referred to Appendix 2.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: sum of bixafen and desmethyl-bixafen, expressed as bixafen			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	~~0.02~~ 0.01 mg/kg in meat and liver ~~0.02~~ 0.01 mg/L in blood plasma	LC-MS/MS	Sam, 2023
Confirmatory	~~0.02~~ 0.01 mg/kg in meat and liver ~~0.02~~ 0.01 mg/L in blood plasma	LC-MS/MS	Sam, 2023

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

5.3.2.8 Other studies/ information

No other studies are required.

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	Kishora, K.S.	2023	Purity analysis of GLOB2111F AG-G0105 Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	Globachem NV
KCP 5.1.2	Ciorga, B.	2023a	Validation of analytical method for the determination of active substance bixafen concentration in aqueous media solutions of the test item GLOB2111F 0064/0023/FA SORBOLAB Research Laboratory LLC GLP Unpublished	N	Globachem NV
KCP 5.1.2	Ciorga, B.	2023b	Validation of analytical method for the determination of active substance bixafen concentrations in aqueous media solutions of the test item GLOB2111F 0064/0035/FA SORBOLAB Research Laboratory LLC GLP Unpublished	N	Globachem NV
KCP 5.1.2	Ballai, C.	2023a	Validation of Analytical Method for the Determination of GLOB2111F from Feeding Solutions FPBSTUDY-272-VAL1 FumoPrep Kft. GLP	N	Globachem NV

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
			Unpublished		
KCP 5.1.2	Ballai, C.	2023b	Validation of Analytical Method for the Determination of GLOB2111F from Surface-treat Solution FPBSTUDY-272-VAL5 FumoPrep Kft. GLP Unpublished	N	Globachem NV
KCP 5.2	Sam, N.	2023	Validation of analytical method for the determination of bixafen and bixafen-desmethyl residues in animal body fluid and tissues by LC-MS/MS. Report No.: AG-G2321 Eurofins Advinus Agrosciences Services India Private Limited GLP Unpublished	N	Globachem NV

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
KCP 5.1.2	Schoening, R.	2006	Analytical method 01012 for the determination of residues of BYF00587 and its metabolite BYF00587-desmethyl in/on plant matrices by HPLC-MS/MS Report No.: 01012 Bayer CropScience AG GLP Unpublished	N	BCS
KCP 5.1.2	Schoening, R.; Willmess, J.	2007	Analytical method 01036 for the determination of residues of BYF00587 and its metabolite BYF00587-desmethyl in/on animal tissues by HPLC-MS/MS	N	BCS

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
			Report No.: 01036 Bayer CropScience AG GLP Unpublished		
~~KCP 5.1.2~~ KCP 5.2	Bardel, P.; Schoening, R.	2006	Analytical method 00983 for the determination of residues of BYF00587 in/on plant matrices by HPLC-MS/MS Report No.: 00983 Bayer CropScience AG GLP Unpublished	N	BCS
KCP 5.1.2 KCP 5.2	Billian, P.; Druskus, M.	2007	Analytical method 01063 for the determination of residues of BYF00587 and its metabolite BYF00587-desmethyl in/on animal tissues, milk and eggs by HPLC-MS/MS Report No.: 01063 Bayer CropScience AG GLP Unpublished	N	BCS
~~KCP 5.1.2~~ KCP 5.2	Brumhard, B.; Freitag, T	2006	Analytical method 00952 for the determination of residues of BYF 00587 in soil by HPLC-MS/MS Bayer CropScience AG, Report No.: 00952, Edition Number: M-281557-01-1 Date: 11.12.2006 GLP, unpublished GLP Unpublished	N	BCS
KCP 5.1.2 KCP 5.2	Krebber, R.; Braune, M.	2008	Analytical method 01073 for the determination of bixafen (BYF 00587) in drinking and surface water by HPLCMS/MS Report No.: 01073 Bayer CropScience AG GLP Unpublished	N	BCS
KCP 5.1.2 KCP 5.2	Class, T	2007	BYF 00587: Analytical method for the determination of BYF 00587 in air PTRL Europe, Ulm, Germany Report No.: P605077505 Bayer CropScience AG	N	BCS

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
			GLP Unpublished		
KCP 5.2	Ballesteros, C.; Portet, M.	2008	Independent laboratory validation of the analytical method 00983 for the determination of residues of BYF 00587 in/on Plant Matrices by HPLC-MS/MS Report No.: MR-08/005 Bayer CropScience S.A., Lyon, France Bayer CropScience AG GLP Unpublished	N	BCS
KCP 5.2	Ballesteros, C.	2007	Independent Laboratory Validation of the Analytical method 01063 for the determination of residues of BYF00587 and its metabolite BYF00587-desmethyl in/on animal tissues, milk and eggs by HPLC-MS/MS Report No.: MR-08/004 Bayer CropScience S.A., Lyon, France Bayer CropScience AG GLP Unpublished	N	BCS

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
KCP XX	Author	YYYY	Title Company Report N Source	Y/N	Owner

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
			GLP/non GLP/GEP/non GEP Published/Unpublished		

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for bixafen

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

Comments of zRMS:	The analytical method code: 0064/0023/FA was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.1. The limit of detection (LOD): 0.027 mg/L and limit of quantification (LOQ): 0.097 mg/L. The method linearity was evaluated at 5 levels. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance bixafen in aqueous media (deionized water, AAP medium, M7 medium). The method is successfully validated and accepted.

Reference:	KCP 5.1.2
Report	Validation of analytical method for the determination of active substance bixafen concentration in aqueous media solutions of the test item GLOB2111F, Ciorga B., 2023a, 0064/0023/FA.
Guideline(s):	Yes (SANTE/2020/12830 rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

~~The method allows for the quantitative determination of the active substance bixafen in M7 and AAP medium solutions of the test item using high performance liquid chromatography with DAD detection. The concentration of the active substance was determined by calibration curves defined by linear functions of the peak areas. The comparison of the UV spectra and the retention times ensured identification of the active substance.~~

~~Sample solution (8 mL) is transferred into a tube and 2 mL of ethyl acetate was added. The samples are shaken on an roller shaker for 30 minutes. After shaking 1 mL of ethyl acetate is collected. The tubes are then placed in a heating block of the sample concentrator to completely evaporate the solvent. 1 mL of methanol is added to each sample and then the contents are mixed on a vortex shaker. The prepared solutions are filtered through a syringe filter into a chromatographic vial and subjected to chromatographic analysis. The extracts are analysed on the day of validation and are not stored.~~

The method is based on the quantitative determination of active substance concentration in aqueous media (deionized water, AAP medium, M7 medium) solutions of the test item using high-performance liquid chromatography with DAD detection.
The concentration of the active substance were determined by a calibration curves defined by a linear functions of the peak areas. Selected peaks of the determined active substance were manually integrated. Identification of active substance was made by comparing the UV spectrums and retention times of standard solution and the test item solution.

26.90 mg of active substance standard (purity 99.91%) was weighed into a tared volumetric flask. The flask was filled to 10 mL with acetonitrile (active substance concentration 2687.579 mg/L) and left in the ultrasonic bath for 10 minutes. Subsequent dilutions of the standard solution were prepared with acetonitrile. Prepared solutions were transferred to chromatographic vials.

Results and discussions

Table A1: Recovery results from method validation of bixafen using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)
Deionized water	Bixafen	0.097	85.0	3.7
		0.965	87.5	2.8
AAP medium		0.097	81.6	2.5
		0.965	91.1	3.8
M7 medium		0.097	86.8	4.8
		0.965	92.1	1.9

Table A2: Characteristics for the analytical method used for validation of bixafen residues in deionised water, M7 and AAP medium solutions.

	Bixafen
Specificity	No signals from other substances with area exceeding 30% of the LOQ area, in place of the active substances peak. The comparison of the UV spectra allows for the identification of active substance.
Calibration (type, number of data points)	r = 0.999 number of data points: 5 Random regression residuals (di) distribution
Calibration range	0.027 mg/L – 2.150 mg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.027 mg/L / 0.097 mg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of bixafen in deionised water, M7 and AAP medium solutions.

Comments of zRMS:	The analytical method code: 0064/0035/FA was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.1. The limit of detection (LOD) was: AAP medium 0.001 mg/L; M7 medium 0.001 mg/L and limit of quantification (LOQ) equals: AAP medium 0.011 mg/L; M7 medium 0.011 mg/L. The method linearity was evaluated at 5 levels.

	The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance bixafen in aqueous media (AAP medium, M7 medium). The method is successfully validated and accepted.

Reference:	KCP 5.1.2
Report	Validation of analytical method for the determination of active substance bixafen concentrations in aqueous media solutions of the test item GLOB2111F, Ciorga B., 2023b, 0064/0035/FA.
Guideline(s):	Yes (SANTE/2020/12830 rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

The method allows for the quantitative determination of the active substance bixafen in M7 and AAP medium solutions of the test item using high-performance liquid chromatography with DAD detection. The concentration of the active substance was determined by calibration curves defined by linear functions of the peak areas. The comparison of the UV spectra and the retention times ensured identification of the active substance.

Sample solution (8 mL) is transferred into a tube and 2 mL of ethyl acetate was added. The samples are shaken on an roller shaker for 30 minutes. After shaking 1 mL of ethyl acetate was collected. The tubes were placed in the heating block of the sample concentrator to completely evaporate the solvent. 1 mL of methanol was added to each sample and then the contents were mixed on a vortex shaker. The prepared solutions were filtered through a syringe filter into a chromatographic vial and subjected to chromatographic analysis. The extracts are analyzed on the day of validation and are not stored.

Results and discussions

Table A3: Recovery results from method validation of bixafen using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)
AAP medium	Bixafen	0.011	94.5	10
		0.111	111.0	6.5
M7 medium		0.011	83.6	11.1
		0.111	116.7	0.8

Table A4: Characteristics for the analytical method used for validation of bixafen residues in M7 and AAP medium solutions.

	Bixafen
Specificity	No signals from other substances with area exceeding 30% of the LOQ

	Bixafen
	area, in place of the active substances peak. The comparison of the UV spectra allows for the identification of active substance.
Calibration (type, number of data points)	r = 0.999 number of data points: 5 Random regression residuals (di) distribution
Calibration range	0.001 mg/L – 0.342 mg/L (calibration curve range for concentrations after extraction 0.004 mg/L – 1.368 mg/L)
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.001 mg/L / 0.011 mg/L

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of bixafen in M7 and AAP medium solutions.

Comments of zRMS:	The analytical method code: FPBSTUDY-272-VAL1 was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.1. The limit of detection (LOD): 0.1 mg/mL. and limit of quantification (LOQ): 0.1 mg/mL. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance bixafen in feeding solutions. The method is successfully validated and accepted.

Reference:	KCP 5.1.2
Report	Validation of Analytical Method for the Determination of GLOB2111F from Feeding Solutions, Ballai C., 2023, FPBSTUDY-272-VAL1.
Guideline(s):	Yes (SANTE/2020/12830 rev.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

The method allows for the quantitative determination of the active substance bixafen in feeding solutions (Sucrose and deionised water in 50:50) of the test item using HPLC with DAD detector.

2 mL of test sample are diluted with Acetonitrile/HPLC water in ration 50:50. The prepared solutions were filtered through a syringe filter into a chromatographic vial and subjected to chromatographic analysis. The extracts are analyzed on the day of validation and are not stored.

Results and discussions

Table A5: Recovery results from method validation of bixafen using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)
Feeding solution (Sucrose and deionised water in 50:50)	Bixafen	0.1	102.6	0.9
		5	101.7	0.74

Table A6: Characteristics for the analytical method used for validation of bixafen residues in Feeding solution

	Bixafen
Specificity	Interference in blank samples ≤ 30% of the LOD.
Calibration (type, number of data points)	$R^2 \geq 0.9998$ number of data points: 7
Calibration range	2 µg/L – 100 µg/L
Assessment of matrix effects is presented	yes
Limit of determination/quantification	0.1 mg/mL

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of bixafen in feeding solutions.

Comments of zRMS:	The analytical method code: FPBSTUDY-272-VAL5 was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.1. The limit of detection (LOD): 0.1 mg/mL. and limit of quantification (LOQ): 0.1 mg/mL. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance bixafen in feeding solutions. The method is successfully validated and accepted.

Reference: KCP 5.1.2

Report: Validation of Analytical Method for the Determination of GLOB2111F Surface-treat Solution, Ballai C., 2023, FPBSTUDY-272-VAL5.

Guideline(s): Yes (SANTE/2020/12830 rev.1)

Deviations: No

GLP: Yes

Acceptability: Yes/~~No/Supplementary~~

Materials and methods

The purpose of this study is to validate a High Performance Liquid Chromatography method with UV detection (HPLC-UV) in order to determine the concentration of GLOB2111F from surface-treat solution (acetone).

2 mL of test sample are diluted with Acetonitrile/HPLC water in ration 50:50. The prepared solutions are filtered through a syringe filter into a chromatographic vial and subjected to chromatographic analysis. The extracts are analyzed on the day of validation and are not stored.

Results and discussions

Table A7: Recovery results from method validation of bixafen using the analytical method

Matrix	Analyte	Fortification level (mg/mL) (*n* = 5)	Mean recovery (%)	RSD (%)
Surface-treat Solution (Acetone)	Bixafen	1	108.9	0.47
		60	107.5	0.64

Table A8: Characteristics for the analytical method used for validation of bixafen residues in Feeding solution

	Bixafen
Specificity	Interference in blank samples ≤ 30% of the LOD.
Calibration (type, number of data points)	$R^2 \geq 1.000$ number of data points: 7
Calibration range	0.5 µg/L – 50 µg/mL
Assessment of matrix effects is presented	yes
Limit of determination/quantification	1 mg/mL

Conclusion

The method was sufficiently validated according to SANTE/2020/12830, Rev.1 and can be used for analytical determination of bixafen in feeding solutions.

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)

Comments of zRMS:	The analytical method code: AG-G2321 was fully validated in term of specificity, linearity, repeatability, accuracy and LOQ according to SANTE/2020/12830, rev.2. The limit of detection (LOD): 0.0025 mg/L and limit of quantification (LOQ): 0.01 mg/L. The method linearity was evaluated at 5 levels. The results of analytical method validation confirm that this method is suitable for the detection, quantification and confirmation of Bixafen and Bixafen-desmethyl in animal body fluid and tissues. The method is successfully validated and accepted.

Reference:	KCP 5.2
Report	Validation of analytical method for the determination of bixafen and bixafen-desmethyl residues in animal body fluid and tissues by LC-MS/MS, Sam N., 2023, AG-G2321.
Guideline(s):	Yes (SANTE/2020/12830, Rev.2)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

Residues of bixafen and desmethyl-bixafen are extracted from animal tissues (meat and liver) and body fluid (blood plasma) using a mixture of acetonitrile/water (80:20%, v/v). The animal tissue samples are further cleaned up using the QuEChERs method, whereas blood plasma was analyzed without further cleanup. The resulting samples are analysed by liquid chromatography with positive-ion Electrospray Ionization (ESI) tandem mass spectrometry (LC-MS/MS). Two MRM transitions are monitored for bixafen, *m/z* 413.8 -> 394.2 (quatification) and *m/z* 413.8 -> 266.2 (confirmation). Similarly, two MRM

transitions were monitored for desmethyl-bixafen: *m/z* 400.1 -> 380.0 (quatification) and *m/z* 400.1-> 360.2 (confirmation).

Chromatographic Conditions for Bixafen and Bixafen-desmethyl

HPLC Conditions:

Instrument : Shimadzu HPLC, Model: SIL-40C X3
Column : Zodiac C18 [150 mm × 4.6 mm × 5µm]
Autosampler temperature : 15°C
Column oven : 30°C
Mobile phase : Solvent A: 0.1% Formic acid in Milli-Q® water
Solvent B: Acetonitrile

Gradient program :

Time (min)	Mobile Phase Composition	
	%A	%B
0.00	90	10
0.30	90	10
3.00	0	100
7.00	0	100
8.00	90	10
10.00	90	10

Run time : 10 minutes
Flow rate : 0.8 mL/min without split
Injection Volume : 10 µL

Mass Spectrometer Conditions:

MS system : Triple Quad API 5500+ System, SCIEX, LC-MS/MS (Triple quadrupole mass spectrometer)
Ionisation type : Electrospray (ESI, TurboIon Spray)
Polarity : Positive
Scan type : MS/MS, Multiple Reaction Monitoring (MRM)
Parameters :

Analyte Monitored	Ions Monitored (*m/z*)	Declustering Potential (DP)	Collision Energy (CE)	Collision Cell Exit Potential (CXP)	Dwell Time (msec)
Bixafen	413.8 >> 394.2*	100	28	10	200
	413.8 >> 266.2**				
Bixafen-desmethyl	400.1 >> 380.0*	100	28	10	200
	400.1 >> 360.2**				

Results and discussions

Table A9: Recovery results from method validation of bixafen and desmethyl-bixafen using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Meat	Bixafen	0.01 (n = 5)	88.8 (413.8 > 394.2)	~~5.3~~ 5.0	The recoveries of all the

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
			107.2 (413.8 > 266.2)	~~4.9~~ 4.7	samples analysed were in the range of 70-120% with %RSD < 20%.
	desmethyl-bixafen	0.01 (n = 5)	101.4 (400.1 > 380.0)	8.1	
			104.4 (400.1 > 360.2)	2.1	
Liver	Bixafen	0.01 (n = 5)	95.4 (413.8 > 394.2)	5.0	
			109.2 (413.8 > 266.2)	~~4.4~~ 4.6	
	desmethyl-bixafen	0.01 (n = 5)	104.6 (400.1 > 380.0)	5.6	
			108.2 (400.1 > 360.2)	2.1	
Plasma	Bixafen	0.01 (n = 5)	97.6 (413.8 > 394.2)	4.9	
			94.2 (413.8 > 266.2)	6.3	
	desmethyl-bixafen	0.01 (n = 5)	101.8 (400.1 > 380.0)	1.9	
			102.8 (400.1 > 360.2)	1.1	

Table A10: Characteristics for the analytical method used for validation of bixafen and desmethyl-bixafen residues in body fluids and tissues

	Bixafen	Desmethyl-bixafen
Specificity	Data were provided for both mass transitions. The samples showed no significant interference (above 30 % of LOQ) at the retention time of the analyte in test medium.	Data were provided for both mass transitions. The samples showed no significant interference (above 30 % of LOQ) at the retention time of the analyte in test medium.
Calibration (type, number of data points)	individual calibration data presented calibration line equation presented linear regression, r>0.999 number of data points: 5 Data have been provided for both transitions.	individual calibration data presented calibration line equation presented linear regression, r>0.999 number of data points: 5 Data have been provided for both transitions.
Calibration range	Accepted calibration range in concentration units: 0.00251 – 0.15063 µg/ml Corresponding calibration range in mass ratio units for the sample: 2.5100 – 150.6300 µg/L in meat, liver and plasma	Accepted calibration range in concentration units: 0.00252 – 0.151245 µg/ml Corresponding calibration range in mass ratio units for the sample: 2.5200 – 151.2450 µg/L in meat, liver and plasma
Assessment of matrix effects is presented	Yes. Matrix matched standards were used.	Yes. Matrix matched standards were used.
Limit of determination/quantification	0.01 mg/L in meat, liver and plasma.	0.01 mg/L in meat, liver and plasma.

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

No new or additional studies have been submitted.