REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: BAS 736 00 F

Product name(s): **Miralon**

Chemical active substance(s):

Fluxapyroxad, 50 g/L
Azoxystrobin, 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(new authorization)

Applicant: BASF

Submission date: 12/2021

Evaluation date: September 2022

Update: December 2022

MS Finalisation date: January 2023

Version history

When	What
12/2021	Initial dRR - BASF DocID 2021/2042572
09/2022	zRMS-PL evaluation
12/2022	Updated version - BASF DocID 2022/2059489
01/2023	zRMS-PL changes as result of MSs comments

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zRMS's comments or conclusions are highlighted in grey colour.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.
Noticed data gaps are: ~~none~~

- since Z-isomer is identified as toxicologically relevant for azoxystrobin (according to Commission Implementing Regulation (EU) No 540/2011), an analytical method for the determination of the Z-isomer in plant protection product BAS 736 00 F is required and should be provided by the applicant. (According to the applicant's statement: analytical method for the z-isomer is in development; study will be provided as soon as available)

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
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)

The plant protection product BAS 736 00 F was not a representative formulation. The product has not been previously evaluated according to Uniform Principles.
Analytical methods for determination of the active substances, impurities and relevance of CIPAC methods in BAS 736 00 F were not evaluated as part of the EU review. Therefore, all relevant data are provided and are considered adequate.

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

The analytical method AFL1000/01 has been developed for analysis of azoxystrobin and fluxapyroxad in the plant protection product BAS 736 00 F.

Comments of zRMS:	The presented, below, HPLC and UHPLC with DAD/UV detection analytical methods have been validated according to EU Guidance SANCO/3030/99 rev.5. Both methods are acceptable for the determination of fluxapyroxad and azoxystrobin in the formulation BAS 736 00 F.

Reference:	CP 5.1.1/1
Report	Validation of the analytical method AFL1000/01: Determination of the Active Ingredients Fluxapyroxad and Azoxystrobin in BAS 736 00 F and Aqueous Solutions of BAS 736 00 F by HPLC and UHPLC, Frohn, D., 2020 report No 848514_1 BASF Ref. 2020/2002656 Authority registration No
Guideline(s):	EC 1107/2009, SANCO/3029/99, SANCO/3030/99 rev.5, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, 2004/10/EC (2004)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Materials and methods

The analytical method AFL1000/01 is applicable to the determination of the content of the active substances azoxystrobin and fluxapyroxad in the EC formulation BAS 736 00 F. The active ingredients are extracted into a water/acetonitrile diluent and analysed by reverse phase HPLC or UHPLC with DAD/UV detection. Both methods can alternatively be used also in production to quantify the active substances.
The amounts of the active substances were calculated using external calibrations with authentic reference items by applying bracketing. The identity of the test item was confirmed by comparing retention times and the UV-spectra of the test item and the reference items.

Approximately 180 mg of BAS 736 00 F is weighted into a 100 mL volumetric flask and diluted in acetonitrile.

Chromatographic Conditions for HPLC:

<table>
<tr><td>Column</td><td>Waters XBridge C18 (100 mm x 4.6 mm; 3.5 µm)</td></tr>
<tr><td>Column temperature</td><td>40 °C</td></tr>
<tr><td>Injection volume</td><td>5 µL</td></tr>
<tr><td>Detection wavelength</td><td>230 nm (Reg.No.:300254 and Reg.No.:5094351)</td></tr>
<tr><td>Flow rate</td><td>1.6 mL/min</td></tr>
<tr><td>Eluent</td><td>A: 1000 mL water + 1mL Formic acid conc.
B: 1000 mL Acetonitrile + 1mL Formic acid conc.</td></tr>
<tr><td>Approx. retention times</td><td>6.05 min. Reg.No.:300254
7.00 min. Reg.No.:5094351</td></tr>
<tr><td>Gradient</td><td>
<table>
<tr><th>Time [min]</th><th>A [%]</th><th>B [%]</th></tr>
<tr><td>0.00</td><td>60</td><td>40</td></tr>
<tr><td>8.00</td><td>60</td><td>40</td></tr>
<tr><td>8.10</td><td>5</td><td>95</td></tr>
<tr><td>13.00</td><td>5</td><td>95</td></tr>
<tr><td>13.10</td><td>60</td><td>40</td></tr>
<tr><td>18.00</td><td>60</td><td>40</td></tr>
</table>
</td></tr>
<tr><td>Dwell Volume</td><td>165 µL (Agilent 1290)</td></tr>
</table>

Chromatographic Conditions for UHPLC:

Column	Waters Acquity BEH C18 (50 mm x 2.1 mm; 1.7 µm)
Column temperature	40 °C
Injection volume	1 µL
Detection wavelength	230 nm (Reg.No.:300254 and Reg.No.:5094351)
Flow rate	0.7 mL/min
Eluent	A: Water + 0.1 % Formic Acid (1000/1 v/v) B: Acetonitrile + 0.1% Formic Acid (1000/1 v/v)
Approx. retention times	1.58 min. Reg.No.:300254 1.80 min. Reg.No.:5094351
Gradient	Time [min] / A [%] / B [%] 0.00 / 60 / 40 2.00 / 60 / 40 2.10 / 5 / 95 3.00 / 5 / 95 3.10 / 60 / 40 4.00 / 60 / 40
Dwell Volume	149 µL (Agilent 1290 Infinity II)

The evaluation of the HPLC and UHPLC data for the determination of precision was carried out by comparison of the peak areas with an authentic external reference items by applying bracketing calibration.
The evaluation of the HPLC and UHPLC data for the determination of accuracy was carried out by comparison of the peak areas with an external calibration series of an authentic reference items by applying linear regression.

Validation - Results and discussions

Identity: the identity of the active substances in BAS 736 00 F was confirmed by comparison of the retention times in combination with the UV spectra resulting from the test item and the authentic reference items.

Specificity: the specificity of the method was confirmed by comparing HPLC and UHPLC chromatograms of blank injections of the solvent, with injections of the reference items fluxapyroxad and azoxystrobin, with injections of the formulation BAS 736 00 F as well as injections of the blank formulation. The chromatograms were free of interfering signals and no co-elution was observed.

Linearity: The linearity of the detector response was determined by preparing a calibration series of the pure reference items. Linearity was demonstrated by preparing six calibration solutions which were injected twice. The correlation coefficient for both active substances were > 0.999 with no force through zero for HPLC and UHPLC.

Accuracy: the evaluation of the accuracy was done by analysing five sample solutions containing the blank formulation fortified with azoxystrobin and fluxapyroxad at concentration levels representing approximately 50%, 100% and 150 % relative to the nominal concentration of the formulation in the sample solution. Each sample was injected twice. For the calculation, total recovery was used. The accuracy was confirmed by the determination of the recovery rate by comparing the found and the expected content.

Precision: the precision of the method was determined by analysing five individual sample weights of the formulation. The acceptability of the %RSD was proven by the Horwitz equation and Horrat value.

Table 5.2- 1: Methods suitable for the determination of active substances azoxystrobin and fluxapyroxad in the plant protection product BAS 736 00 F

	Azoxystrobin (Reg.No. 300254)	**Fluxapyroxad (Reg.No. 5094351)**
year	2020	
Principle of method	RP HPLC or RP UHPLC with UV detection	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Range: 27.1 – 256.6 mg/L Corresponding to 1.3-12.5% w/w of declared content in the formulation (=6.8%w/w) HPLC: Correlation coefficient: 1.0000 Intercept: -0.887 Slope: 6.24E-07 UHPLC: Correlation coefficient: 1.0000 Intercept: 0.173 Slope: 1.29E-06	Range: 20.1 – 170.7 mg/L Corresponding to 1.0-8.7% w/w of declared content (=4.6%w/w) HPLC: Correlation coefficient: 0.9998 Intercept: -1.124 Slope: 6.75E-07 UHPLC: Correlation coefficient: 0.9998 Intercept: -0.028 Slope: 1.37E-06
zRMS: Range appropriate to the nominal concentration of the analyte >> ± 20%. Duplicate determinations at ~~3~~ 6 concentrations – independently weighed samples; R^2>0.98 (r>0.99) **Acceptable**		
Precision – Repeatability Mean n = 5 (%RSD)	HPLC: %RSD= 0.51 $\%RSD_R$= 3.00 $\%RSD_r$ = 2.01 Horrat, H_r = %RSD / %RSDr H_r = 0.25 (Hr < 1) UHPLC: %RSD= 0.49 $\%RSD_R$= 3.00 $\%RSD_r$ = 2.01 Horrat, H_r = %RSD / %RSDr H_r = 0.24 (Hr < 1)	HPLC: %RSD=0.51 $\%RSD_R$= 3.18 $\%RSD_r$= 2.13 Horrat, H_r = %RSD / %RSDr H_r = 0.24 (Hr < 1) UHPLC: %RSD= 0.58 $\%RSD_R$= 3.18 $\%RSD_r$ = 2.13 Horrat, H_r = %RSD / %RSDr H_r = 0.27 (Hr < 1)
zRMS: 5 independently weighted formulation sample determinations %RSD < %RSDr Horrat ratio: Hr<1 **Acceptable**		
Accuracy **n = 3x5=15** for each HPLC and UHPLC **(% Recovery)**	HPLC: Level approx. 50 % of declared content: main recovery: 100.1 % Level approx. 100 % of declared content: main recovery: 100.7 % Level approx. 150 % of declared content: main recovery: 100.8 % UHPLC:	HPLC: Level approx. 50 % of declared content: main recovery: 98.6 % Level approx. 100 % of declared content: main recovery: 99.7 % Level approx. 150 % of declared content: main recovery: 100.4 % UHPLC:

	Azoxystrobin (Reg.No. 300254)	**Fluxapyroxad (Reg.No. 5094351)**
	Level approx. 50 % of declared content: main recovery: 100.2 % Level approx. 100 % of declared content: main recovery: 100.0 % Level approx. 150 % of declared content: main recovery: 100.4 %	Level approx. 50 % of declared content: main recovery: 99.5 % Level approx. 100 % of declared content: main recovery: 99.3 % Level approx. 150 % of declared content: main recovery: 100.3 %
zRMS: 3 independent recovery determinations using the standard addition method, ~~however two fortified concentration levels are outside the required range between 90-110% of the target concentration in the formulation, but one fortification level is within above renge, therefore accuracy can be accepted~~. All recoveries are between 90-110% for both active substances. **Acceptable**		
Interference/ Specificity	none/given	none/given
zRMS: Specificity chromatograms are provided by the applicant in study report. These demonstrate no analyte interferences and that method is specific to azoxystrobin and fluxapyroxad in the plant protection product BAS 736 00 F		
Comment	suitable	suitable

Conclusion

The validation data of method AFL1000/01 with respect to accuracy, precision, linearity and specificity prove that the method is suitable to the determination of the content of the active substances azoxystrobin and fluxapyroxad in the EC formulation BAS 736 00 F.
Both methods HPLC and UHPLC, can alternatively be used also in production to quantify the active substances in the plant protection product.

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:

Azoxystrobin and fluxapyroxad both contain toluene as relevant impurity. The maximum allowed level in the technical material is 2 g/kg and 0.6 g/kg for azoxystrobin technical and fluxapyroxad technical respectively, which means 0.19 g/L (approx. 0.018%) in the formulation BAS 736 00 F.

The analytical AFL1012/01 method has been developed to determine the content of toluene in the EC formulation BAS 736 00 F. The following analytical method has not previously been reviewed and is provided in support of this assessment.

Comments of zRMS:	The presented, below, GC with MS detection analytical method has been validated according to EU Guidance SANCO/3030/99 rev.5. The method is acceptable for the determination of relevant impurity toluene in the formulation BAS 736 00 F at LOQ=0.01%.

Reference:	CP 5.1.1/2
Report	Validation of the Analytical Method AFL1012/01: "Determination of Toluene in BAS 736 00 F containing Fluxapyroxad and Azoxystrobin by GC-MS", Schubring, M., 2020 report No 885326_1 BASF Ref. 2020/2033569 Authority registration No
Guideline(s):	CIPAC Guidelines on method validation, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Reference:	CP 5.1.1/3
Report	Additional Validation of the Analytical Method AFL1012/01: "Determination of Toluene in BAS 736 00 F containing Fluxapyroxad and Azoxystrobin by GC-MS", Schubring, M., 2021 report No 921927_1 BASF Ref. 2021/2022815 Authority registration No
Guideline(s):	CIPAC Guidelines on method validation, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Materials and methods

The analyte is extracted in acetonitrile and separated from other components by gas chromatography using a RTX-200 capillary column. The analyte is detected using a MS detector and quantified with external standard by applying linear regression. The specific response ratio of the sample is compared with those of the standard of known quantity.

Approximately 500 mg of the test item is weighted into a 25 mL volumetric flask and dissolved in acetonitrile. Aliquots of the final solutions are injected in duplicate into the GC-MS system.

The evaluation of the GC-MS analyses is carried out by comparison of the peak areas with an external calibration series of an authentic reference item by applying linear regression.

Chromatographic Conditions:

Column	Rtx-200, 30m x 0.32mm, 1.5 µm		
Injector temperature	250 °C		
MS transferline temperature	250 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold Time [min]
	-	120	4
	20	250	4
Carrier gas	Helium		
Detector	MSD		
Split ratio	10:1		
Column flow	1.5 mL/minute (constant flow)		
Injection volume	1.5 µL		
Analysis time	14.5 min		
Source temperature	230 °C		
Quad temperature	150 °C		
Solvent delay	3 min		
MS off	After 5 min		

Target compound	Retention time [min]	m/z [quantifier]	m/z [qualifier]
Reg.No.:4005250	3.59	91	92

Validation - Results and discussions

Identity: the identity of toluene (Reg.No. 4005250) in BAS 736 00 F is confirmed by comparing the retention time in combination with the MS-spectra of the test item fortified with the reference item.

Specificity: chromatograms of the test item, the reference substance, the solvent blank, the test item fortified and the blank formulation were measured and no interferences were detected.

Linearity: the linearity of the detector response is determined by preparing a calibration series of the pure reference item. Linearity is demonstrated by preparing five calibration solutions which are injected twice. Data evaluation confirmed a linear detector response. The correlation coefficient is 0.999 with no force through zero.

Accuracy: the evaluation of the accuracy is done by analyzing for each concentration level five sample solutions containing BAS 736 00 F fortified with a concentration of approximately 0.010%, 0.018% or 0.022% toluene relative to the nominal weight in concentration of the formulation. Each sample is injected twice. For the calculation, marginal recovery was used. The accuracy is confirmed by the determination of the recovery rate by comparing the found and the expected content. At all level, mean recovery was found to be between 75 % and 125 %.

Precision: the content of toluene in BAS 736 00 F was smaller than the limit of quantification, i.e., smaller than 0.010%. Therefore, the precision was determined using the 0.018 % accuracy samples and calculating the relative standard deviation of the recovery rates. The acceptability of the %RSD was proven by the Horwitz equation and Horrat value.

Limit of quantification (LOQ): the LOQ of the method is in accordance with the lowest accuracy level. This means, that the LOQ corresponds to the 0.010 % concentration.

Table 5.2- 2: Methods suitable for the determination of the relevant impurity toluene in the plant protection product BAS 736 00 F

	Relevant impurity toluene max. content in BAS 736 00 F: 0.19 g/L[1]
year	2020
Principle of method	GC with MS detection
Linearity (linear between mg/L) n=5 (correlation coefficient, expressed as r)	Range: 1.6 mg/L to 6.0 mg/L (equivalent to 80 mg/kg to 300 mg/kg in the formulation) Correlation coefficient: R^2= 0.997 (r=0.99861) Intercept= -7845.57 Slope= 3.91E+04
Precision – Repeatability Mean n = 5 (%RSD)	@ 0.018 % level %RSD= 3.86 $\%RSD_R$= 7.49 $\%RSD_r$ = 5.02 Horrat, H_r = %RSD / %RSDr H_r = 0.77 (Hr < 1)
Accuracy n = 15 (5x3) (% Recovery)	@ 0.010 % level, mean recovery is 86.3 % @ 0.018 % level, mean recovery is 86.3 % @ 0.022 % level, mean recovery is 85.7 % All recoveries between 75-125%, acceptable For all level, marginal recovery was used
Interference/ Specificity	none/given
LOQ	0.010 %
Comment	suitable

Conclusion

The validation data of method AFL1012/01 with respect to accuracy, precision, linearity and specificity prove that the method is suitable to the determination of the content of the relative impurity toluene in the formulation BAS 736 00 F.

An analytical method for the determination of the z-isomer of azoxystrobin in the formulation BAS 736 00 F is in development and will be delivered as soon as available.

[1] Max. content of toluene is reported by the applicant considering the content of toluene in fluxapyroxad ≤0.6g/kg according to Review report for the active substance fluxapyroxad: SANCO/10692/2012 Rev 2, 25 March 2021. However according to Regulation (EU) No 540/2011 (version 01/11/2022) the maximum content of toluene is 1 g/kg as implemented with Regulation (EU) No 589/2012.
Taking into account that reported max. content of toluene reflects a worse case, it may be used in this submission.

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

No methods are required for co-formulants or components of co-formulants. These ingredients are not considered toxicologically or ecotoxicologically relevant at the concentrations present in the formulation.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of azoxystrobin and fluxapyroxad in the same preparation.
There is no CIPAC method for the determination of fluxapyroxad in solo formulations.
There is no CIPAC method for the determination of azoxystrobin in EC 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 fluxapyroxad and azoxystrobin 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.

Fluxapyroxad

Table 5.2- 3: Validated methods for the generation of pre-authorization data of fluxapyroxad and metabolites in plant and animal matrices

Component considered for residue definition: Fluxapyroxad, M700F002, M700F008				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products				
Wheat – whole plant, grain, straw Soya bean – seed, refined oil Field pea – dried seed Potato – tuber, crisps Onion – bulb Cauliflower – head Avocado – fruit Lemon – fruit Apple – fruit, juice Tomato – fruit Grape – fruit, raisin Coffee – green bean Beer	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed This method can also be used for post-registration monitoring (Fluxapyroxad), see section 5.3.2.
Tomato – fruit Lemon – fruit Onion – bulb Soya bean – seed Wheat – grain,	Additional (independent) validation	0.01 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0137/01 EU agreed

Component considered for residue definition: Fluxapyroxad, M700F002, M700F008				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
straw (Residues)				This method can also be used for post-registration monitoring (Fluxapyroxad), see section 5.3.2.
		0.01 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074614 Method L0137/01 EU agreed
Animal products, food of animal origin				
Cow – muscle, kidney, liver, fat, milk, skim milk, cream Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg (muscle, kidney, fat, liver) 0.001 mg/kg (milk, skim milk, cream, egg)	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L0140/02 EU agreed This method can also be used for post-registration monitoring (Fluxapyroxad), see section 5.3.2.
Cow – muscle, liver, milk Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg (muscle, liver) 0.001 mg/kg (milk, egg)	HPLC-MS/MS	Macdougall J. 2009 BASF DocID 2009/1074797 Method L0140/01 EU agreed
Cow – muscle, liver, milk Hen – egg (Residues)	Additional (independent) validations	0.01 mg/kg (muscle, liver) 0.001 mg/kg (milk, egg)	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed This method can also be used for post-registration monitoring (Fluxapyroxad), see section 5.3.2.

Table 5.2- 4: Validated methods for the generation of pre-authorization data of metabolite M700F048 in plant and animal matrices

Component considered for residue definition: M700F048				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products				
Wheat – whole pant, grain, straw Soya bean – seed, refined oil Field pea – dried seed Potato – tuber, crisps Onion – bulb Cauliflower – head Avocado – fruit Lemon – fruit Apple – fruit, juice Tomato – fruit Grape – fruit, raisin Coffee – green bean Beer (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed
Animal products, food of animal origin				
Cow – muscle, kidney, liver, fat, milk, skim milk, cream Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg (muscle, kidney, fat, liver) 0.001 mg/kg (milk, skim milk, cream, egg)	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L0140/02 EU agreed
Cow – muscle, liver, milk Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg (muscle, liver) 0.001 mg/kg (milk, egg)	HPLC-MS/MS	Macdougall J. 2009 BASF DocID 2009/1074797 Method L0140/01 EU agreed

Table 5.2- 5: Validated methods for the generation of pre-authorization data of fluxapyroxad and metabolites in soil and water matrices

Component considered for residue definition: fluxapyroxad, M700F001, M700F002				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS UPLC-MS/MS	Zangmeister W. 2009 Zangmeister W. 2010 BASF DocID 2008/1063799 BASF DocID 2010/1043659 Method L0092/03 EU agreed This method can also be used for post-registration monitoring (Fluxapyroxad), see section 5.3.2.
Water (Drinking and surface water)	Primary (VAL) Confirmatory method not necessary (two mass transitionsfor confirmation)	0.03 µg/L	HPLC-MS/MS	Zangmeister W. 2009 BASF DocID 2009/1069396 Method L0143/01 EU agreed This method can also be used for post-registration monitoring (Fluxapyroxad), see section 5.3.2.

Table 5.2- 6: Validated methods for the generation of pre-authorization data of metabolite M700F003 in soil

Component considered for residue definition: M700F003				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS UPLC-MS/MS	Zangmeister W. 2009 Zangmeister W. 2010 BASF DocID 2008/1063799 BASF DocID 2010/1043659 Method L0092/03 EU agreed

Table 5.2- 7: Validated methods for the generation of pre-authorization data of metabolite M700F007 in water

Component considered for residue definition: M700F007				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.03 µg/L	HPLC-MS/MS	Zangmeister W. 2009 BASF DocID 2009/1069396 Method L0143/01 EU agreed

Table 5.2- 8: Validated methods for the generation of pre-authorization data of fluxapyroxad in air

Component considered for residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Air (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.06 µg/m^3	HPLC-MS/MS UPLC-MS/MS	Zangmeister W. 2009 BASF DocID 2009/1069395 Method L0142/01 EU agreed

Table 5.2- 9: Validated methods for the generation of pre-authorization data of fluxapyroxad in bee-related matrices

Component considered for residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Bee-related matrices Flowers, Nectar surrogate, Pollen (Ecotoxicology)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Obermann M. 2020 BASF DocID 2020/2000481 Method L0372/02 New method, not peer-reviewed CP 5.1.2/1 (A 2.1.1.1, Appendix 2)

Azoxystrobin

Table 5.2- 10: Validated methods for the generation of pre-authorization data in plant matrices

Component of residue definition: azoxystrobin and R230310				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants: mandarin, cabbage, wheat grain, wheat straw, wheat flour, sunflower seed, beer	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Chaggar, S., 2004 Syngenta File No. ICI5504/2636 Method RAM 305/03 EU agreed

Table 5.2- 11: Validated methods for the generation of pre-authorization data in soil

Component of residue definition: azoxystrobin, R230310, R234886, R410553, R402173				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (Environmental fate)	Primary (VAL)	0.02 mg/kg for azoxystrobin, R230310 and R2334886; 0.01 mg/kg for R410553, R402173	LC-MS/MS	Johnson, R.I., et al., 2000 Syngenta File No. ICI5504/0751 Method RAM 269/03 EU agreed The method can also be used for post-registration monitoring (Azoxystrobin), see section 5.3.3.
	Confirmatory for azoxystrobin, R230310 and R234886	0.02 mg/kg	HPLC-UV	Johnson, R.I., et al., 2000 Syngenta File No. ICI5504/0751 Method RAM 269/03 EU agreed The method can also be used for post-registration monitoring (Azoxystrobin), see section 5.3.3.
	Confirmatory for R410553 and R402173	0.01 mg/kg	GC-MSD	Johnson, R.I., et al., 2000 Syngenta File No. ICI5504/0751 Method RAM 269/03 EU agreed

Table 5.2- 12: Validated methods for the generation of pre-authorization data in water and air

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Water (Surface, Ground and drinking water)	Primary (VAL) Confirmatory method not necessary (one quantification ion and two qualifier ions)	0.1 µg/L	GC-MSD	Robinson, N.J., 2000 Syngenta File No. ICI5504/0758 Method RAM 358/01 EU agreed The method can also be used for post-registration monitoring (Azoxystrobin), see section 5.3.3.

Table 5.2- 13: Validated methods for the generation of pre-authorization data in air

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Air	Primary (VAL) Confirmatory method not necessary (one quantification ion and two qualifier ions)	0.003 mg/m^3	GC-MSD	Crawford, N., 2001 Syngenta File No. ICI5504/0011 Method RAM 376/01 EU agreed The method can also be used for post-registration monitoring (Azoxystrobin), see section 5.3.3.

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)

Suitable methods can be found in point 5.2.1 above (KCP 5.1.1).

5.3.2 Description of analytical methods for the determination of residues of fluxapyroxad (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 com pliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Fluxapyroxad	0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Plant, high acid content		0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Plant, high oil content		0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Muscle	Fluxapyroxad	~~0.02~~ 0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Milk		~~0.02~~ 0.001mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Eggs		~~0.02~~ 0.001mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Fat		~~0.05~~ 0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Liver, kidney		~~0.02~~ 0.01 mg/kg	Reg. (EU) No ~~2021/644~~ 2022/1324
Soil (Ecotoxicology)	Fluxapyroxad	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Fluxapyroxad	0.1 µg/L	General limit for drinking water
Surface water	Fluxapyroxad	35.9 µg/L	NOEC *Pimephales promelas*

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
(Ecotoxicology)			
Air	Fluxapyroxad	0.04 mg/kg bw/day 5 310 000 µg/m^3	AOEL sys LC_{50} inhal.
Tissue (muscle, liver, kidney)	Fluxapyroxad	0.01 mg/kg	Common limit
Body fluids	Fluxapyroxad	0.01 mg/kg	Common limit

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 fluxapyroxad in plant matrices is given in the following tables. No new studies are submitted.

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: fluxapyroxad				
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 (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class T.,Jooss S. 2009 BASF DocID 2009/1074618 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High acid content	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class T.,Jooss S. 2009 BASF DocID 2009/1074618 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2
High oil content	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class T.,Jooss S. 2009 BASF DocID 2009/1074618 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class T.,Jooss S. 2009 BASF DocID 2009/1074618 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2
Difficult (if required, depends on intended use)	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS	Lehmann, A., Mackenroth, C. 2009 BASF DocID 2009/1074617 Method L0137/01 EU agreed This method can also be used for data generation, see section 5.2.2

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	In the metabolism studies (tomato, wheat) submitted and reviewed during Annex I inclusion process, extraction effiency of the analytical methods was tested.
Not required, because:	Three procedures were compared: a three step extraction method (L0137/01) with methanol / water (1 / 1, v / v), a three step extraction method (L0076/03) with methanol / water / 2N HCl (70 / 25 / 5, v / v / v) and a three step extraction (Multi-residue method S19) with acetone / water (2 / 1, v / v). In general all extraction methods were comparable to the extraction method used for metabolism investigations in the current study concerning the amount and composition of residues.

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 fluxapyroxad in animal matrices is given in the following tables. No new studies were submitted.

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

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk, cream	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L140/02 EU agreed This method can also be used for data generation, see section 5.2.2
Milk	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed This method can also be used for data generation, see section 5.2.2
Eggs	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L140/02 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed This method can also be used for data generation, see section 5.2.2

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Muscle	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009a BASF DocID 2009/1074613 Method L140/02 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed This method can also be used for data generation, see section 5.2.2
Fat	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009a BASF DocID 2009/1074613 Method L140/02 EU agreed This method can also be used for data generation, see section 5.2.2
Kidney	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009a BASF DocID 2009/1074613 Method L140/02 EU agreed This method can also be used for data generation, see section 5.2.2
Liver	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009a BASF DocID 2009/1074613 Method L140/02 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed This method can also be used for data generation, see section 5.2.2

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	In the metabolism studies (poultry, goat) submitted and reviewed during Annex I inclusion process, extraction effiency of the analytical methods was tested.
Not required, because:	The extractability was tested using BASF method L0140/02. The extraction was performed with a mixture of acetonitrile/water (80 : 20). The amounts of residues extracted with method L0140/02 were in the same range as the amounts of residues extracted within the current study for metabolism investigations.

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 fluxapyroxad in soil is given in the following tables. No new studies are submitted.

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

Component of residue definition: fluxapyroxad			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.001 mg/kg	HPLC-MS/MS UPLC-MS/MS	Zangmeister W. 2009 Zangmeister W. 2010 BASF DocID 2008/1063799 BASF DocID 2010/1043659 Method L0092/03 EU agreed This method can also be used for data generation, see section 5.2.2

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 fluxapyroxad in surface and drinking water is given in the following tables. For the detailed valuation of the new independent laboratory validation study, please refer to Appendix 2.

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

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Water	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.03 µg/L	HPLC-MS/MS	Zangmeister W. 2009b BASF DocID 2009/1069396 Method L0143/01 EU agreed This method can also be used for data generation, see section 5.2.2
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.03 µg/L	HPLC-MS/MS	Perez R., Perez S. 2011 BASF DocID 2011/7001254 Method L0143/01 New method, not peer-reviewed CP 5.2/1 (A 2.1.2.4, Appendix 2)

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 fluxapyroxad in air is given in the following tables. No new studies are submitted.

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

Component of residue definition: fluxapyroxad			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.06 µg/m^3	HPLC-MS/MS UPLC-MS/MS	Zangmeister W. 2009 BASF DocID 2009/1069395 Method L0142/01 EU agreed This method can also be used for data generation, see section 5.2.2

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 fluxapyroxad in body fluids and tissues is given in the following table. For the detailed valuation of the new body fluids method validation, please refer to Appendix 2.

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

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Body fluids	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S., Djedovic S., 2016, BASF DocID 2016/1217548 L0352/01 New method, not peer-reviewed CP 5.2/2 (A 2.1.2.6, Appendix 2)

Component of residue definition: fluxapyroxad				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Muscle	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L140/02 EU agreed
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed
Kidney	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L140/02 EU agreed
Liver	Primary (VAL) Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Hopf, B., Mackenroth, C. 2009 BASF DocID 2009/1074613 Method L140/02 EU agreed
	ILV Confirmatory method not necessary (two mass transitions for confirmation)	0.01 mg/kg	HPLC-MS/MS	Class, T., Jooss, S. 2009 BASF DocID 2009/1074618 Method L0140/02 EU agreed

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

There are no additional European requirements for formulated products.

5.3.3 Description of analytical methods for the determination of residues of azoxystrobin (KCP 5.2)

5.3.3.1 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 5.3-10: 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	azoxystrobin	0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Plant, high acid content		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Plant, high oil content		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Muscle	azoxystrobin	0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Milk		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Eggs		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Fat		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Liver, kidney		0.01 mg/kg	Reg. (EU) ~~2019/552~~ 2022/476
Soil	azoxystrobin	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	azoxystrobin	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	azoxystrobin	9.54 µg/L	NOEC *Mysidopsis bahia*, EFSA Journal 2010; 8 (4):1542
Air	azoxystrobin	0.2 mg/kg bw/day	AOEL sys, EFSA Journal 2010; 8 (4):1542
Tissue (meat or liver)	azoxystrobin	0.1 mg/kg	Classified as T , EFSA Journal 2010; 8 (4):1542
Body fluids		0.05 µg/mL	Classified as T , EFSA

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			Journal 2010; 8 (4):1542

5.3.3.2 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 azoxystrobin in plant matrices is given in the following tables.

Table 5.3-11: 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: azoxystrobin				
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 (VAL)	0.01 mg/kg	LC-MS/MS	Robinson et al., 1999 MET2001-150 Method RAM 305/02 EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Croucher, A., 2002 Syngenta File No. ICI5504/1336 Method RAM 305/02 EU agreed
	Confirmatory	0.01 mg/kg	LC-MS/MS	Chaggar, S., 2004 Syngenta File No. ICI5504/2636 Method RAM 305/03 EU agreed
	Multi-method Validation DFG S19 (L 00.00-34)	0.01 mg/kg	DFG-S19	Klimmek, S., 2004 Syngenta File No. ICI5504/2766 EU agreed
	ILV for the multi-method DFG S19 (L 00.00-34)	0.01 mg/kg	DFG-S19	Lakaschus, S., 2005 Syngenta File No. ICI5504/2948 EU agreed
High acid content	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Robinson et al., 1999 MET2001-150 Method RAM 305/02 EU agreed
	Confirmatory	0.01 mg/kg	LC-MS/MS	Chaggar, S., 2004 Syngenta File No. ICI5504/2636 Method RAM 305/03 EU agreed
High oil content	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Robinson et al., 1999 MET2001-150 Method RAM 305/02 EU agreed
	Confirmatory	0.01 mg/kg	LC-MS/MS	Chaggar, S., 2004 Syngenta File No. ICI5504/2636 Method RAM 305/03 EU agreed

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/high starch content (dry)	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Robinson et al., 1999 MET2001-150 Method RAM 305/02 EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Croucher, A., 2002 Syngenta File No. ICI5504/1336 Method RAM 305/02 EU agreed
	Confirmatory	0.01 mg/kg	LC-MS/MS	Chaggar, S., 2004 Syngenta File No. ICI5504/2636 Method RAM 305/03 EU agreed
	Multi-method Validation DFG S19 (L 00.00-34)	0.01 mg/kg	DFG-S19	Klimmek, S., 2004 Syngenta File No. ICI5504/2766 EU agreed
	ILV for the multi-method DFG S19 (L 00.00-34)	0.01 mg/kg	DFG-S19	Lakaschus, S., 2005 Syngenta File No. ICI5504/2948 EU agreed
Difficult (if required, depends on intended use)	Primary (VAL)			Not required for intended GAP

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Wilkinson, Hepburn, Joseph, 1994, RIP9600103; EU agreed
Not required, because:	Studies about metabolism in different plant matrices are provided in the DAR (vol. 3 B.7.1.1.1). In the study of Wilkinson, 1994 (RIP9600103), approx. 90% of TRR are extracted from wheat samples by acetonitrile/water.

5.3.3.3 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 azoxystrobin in animal matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Richards, S., 2002 Report No. RJ3350B MET2003-158 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.1 Appendix 2)*
	ILV	0.01 mg/kg	LC-MS/MS	Atkinson, 2003 Report No. CEMR-1907 MET2003-159 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.2 Appendix 2)*
	Confirmatory	0.001 mg/kg	GC-NPD	Ryan, J., Sapiets, A., 1996 Syngenta File No. ICIA5504 and R230310 Method RAM 255/03 EU agreed
	ILV	0.001 mg/kg	GC-NPD	EU agreed EFSA Journal 2012; 11 (12):3497
Eggs	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Richards, S., 2002 Report No. RJ3350B MET2003-158 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.1 Appendix 2)*
	ILV			Not required
	Confirmatory	0.01 mg/kg	GC-NPD	Ryan, J., Sapiets, A., 1996 Syngenta File No. ICIA5504 and R230310 Method RAM 255/03 EU agreed
	ILV	0.01 mg/kg	GC-NPD	EU agreed EFSA Journal 2012; 11 (12):3497
Muscle	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Richards, S., 2002 Report No. RJ3350B

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				MET2003-158 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.1 Appendix 2)*
	ILV	0.01 mg/kg	LC-MS/MS	Atkinson, 2003 Report No. CEMR-1907 MET2003-159 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.2 Appendix 2)*
	Confirmatory	0.01 mg/kg	GC-NPD	Ryan, J., Sapiets, A., 1996 Syngenta File No. ICIA5504 and R230310 Method RAM 255/03 EU agreed
	ILV	0.01 mg/kg	GC-NPD	EU agreed EFSA Journal 2012; 11 (12):3497
Fat	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Richards, S., 2002 Report No. RJ3350B MET2003-158 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.1 Appendix 2)*
	ILV			Not required
	Confirmatory	0.01 mg/kg	GC-NPD	Ryan, J., Sapiets, A., 1996 Syngenta File No. ICIA5504 and R230310 Method RAM 255/03 EU agreed
	ILV	0.01 mg/kg	GC-NPD	EU agreed EFSA Journal 2012; 11 (12):3497
Kidney, liver	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Richards, S., 2002 Report No. RJ3350B MET2003-158 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.1 Appendix 2)*
	ILV			Not required
	Confirmatory (only liver)	0.01 mg/kg	GC-NPD	Ryan, J., Sapiets, A., 1996 Syngenta File No. ICIA5504 and R230310

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Method RAM 255/03 EU agreed
	ILV (only liver)	0.01 mg/kg	GC-NPD	EU agreed EFSA Journal 2012; 11 (12):3497

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 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Ryan, 1996; EU agreed
Not required, because:	In the study of Ryan, 1996, also radiolabeled liver samples from a metabolism study are extracted (DAR vol. 3 B.7.9.1) The extraction efficiency was 90 % using acetonitrile as extraction solvent.

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

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

Table 5.3-15: Validated methods for soil (if appropriate)

Component of residue definition: azoxystrobin			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL)	0.02 mg/kg	LC-MS/MS	Johnson, R.I., et al., 2000 Syngenta File No. ICI5504/0751 Method RAM 269/03 EU agreed
Confirmatory	0.02 mg/kg	HPLC-UV	Johnson, R.I., et al., 2000 Syngenta File No. ICI5504/0751 Method RAM 269/03 EU agreed

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

An overview on the acceptable methods and possible data gaps for analysis of azoxystrobin in surface and drinking water is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-16: Validated methods for water (if appropriate)

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water and surface water	Primary (VAL)	0.1 µg/L	GC-MSD	Robinson, N.J., 2000 Syngenta File No. ICI5504/0758 Method RAM 358/01 EU agreed
	Confirmatory	0.05 µg/L	LC-MS/MS	Amic S., 2012 Syngenta File No. ICI5504_11490 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.4.1 Appendix 2)*
	ILV	0.05 µg/L	LC-MS/MS	Brown D., 2019 Report RES-00193 ASB2019-16220 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.4.2 Appendix 2)*

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

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

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

Table 5.3-17: Validated methods for air (if appropriate)

Component of residue definition: azoxystrobin			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary Confirmatory method not necessary (one quantification ion and two qualifier ions)	0.003 mg/m^3	GC-MSD	Crawford, N., 2001 Syngenta File No. ICI5504/0011 Method RAM 376/01 EU agreed

5.3.3.7 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 azoxystrobin in body fluids and tissues is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-18: Methods for body fluids and tissues (if appropriate)

Component of residue definition: azoxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Body fluids (plasma)	~~Primary (VAL)~~	~~0.05 µg/mL~~	~~HPLC-UV~~	~~Hall, M.C., 1999~~ ~~Method CTL/R/1401~~ ~~Syngenta File No. ICI5504/0236~~ ~~EU agreed~~
	~~Confirmatory~~	~~0.05 µg/mL~~	~~LC-MS~~	~~Hall, M.C., 1999~~ ~~Method CTL/R/1401~~ ~~Syngenta File No. ICI5504/0236~~ ~~EU agreed~~
	VAL	0.01 µg/mL	LC-MS/MS	Gemrot, F., 2011 Report S10-03815 ICI5504/11467 New method, not peer-reviewed
Tissues (Muscle and liver)	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Richards, S., 2002 Report No. RJ3350B MET2003-158 Method RAM 399/01 New method, not peer-reviewed dRR Syngenta July 2018 *CP 5.2 (A 2.2.2.2.1 and A 2.2.2.6 Appendix 2)*
	Confirmatory	0.01 mg/kg	GC-NPD	Ryan, J., Sapiets, A., 1996 Syngenta File No. ICIA5504 and R230310 Method RAM 255/03 EU agreed

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

5.3.3.8 Other studies/ information

New methods that are not EU peer-reviewed are published in Registration Report of the Product: AMISTAR GOLD (A18253A) to Germany due to the national assessment by the applicant Syngenta Agro GmbH (30/07/2014 Submission date) in July 2018 (dRR Syngenta July 2018).

Appendix 1 Lists of data considered in support of the evaluation

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 5.1.1/1	Frohn, D.	2020	Determination of the Active Ingredients Fluxapyroxad and Azoxystrobin in BAS 736 00 F and Aqueous Solutions of BAS 736 00 F by HPLC and UHPLC 2020/2002656 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/2	Schubring, M.	2020	Validation of the Analytical Method AFL1012/01: "Determination of Toluene in BAS 736 00 F containing Fluxapyroxad and Azoxystrobin by GC-MS" 2020/2033569 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/3	Schubring, M.	2021	Additional Validation of the Analytical Method AFL1012/01: "Determination of Toluene in BAS 736 00 F containing Fluxapyroxad and Azoxystrobin by GC-MS" 2021/2022815 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.2/1	Obermann, M.	2020	Validation of Analytical Method L0372/02 for the determination of BAS 750 F (Reg.No.5834378), BAS 500 F (Reg.No.304428) and BAS 700 F (Reg.No.5094351) in bee-related matrices by HPLC-MS/MS 2020/2000481 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.2/1	Perez, R., Perez, S.	2011	Independent laboratory validation of BASF analytical method L0143/01: Validation of analytical method L0143/01: Determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 in water by HPLC/MS-MS 2011/7001254 ADPEN Laboratories Inc., Jacksonville FL, United States of America yes Unpublished	No	BASF
KCP 5.2/2	Richter, S., Djedovic, S.	2016	Validation of BASF analytical method L0352/01 for the determination of BAS 700 F (Fluxapyroxad) in body fluids 2016/1217548 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/6	Brown, D.	2019	Azoxystrobin - Independent laboratory validation of analytical method GRM057.01A for the determination of residues of azoxystrobin and its metabolite R234886 in water Report RES-00193, ASB2019-16220 yes Unpublished	No	SYN

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

BAS 736 00 F is a new product, no product data have been evaluated previously.

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	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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	Verte-brate 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 fluxapyroxad

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

A 2.1.1.1 Analytical method L0372/02 for the determination of fluxapyroxad in bee-related matrices

A 2.1.1.1.1 Method validation 1

Comments of zRMS:	Analytical method for the determination of fluxapyroxad (BAS 700 F) in in bee-related matrices (flowers, nectar surrogate and pollen) by LC-MS/MS is fully validated according to SANCO/3029/99 rev. 4 (experimental completion date: July 2019) with a LOQ of 0.01 mg/kg and acceptable according to SANCO/825/00 rev. 8.1 and also according to SANTE/2020/12830 rev. 1 as the minimum requirements for existing methods as mentioned in SANTE/2020/12830 rev. 1 are fulfilled

Reference:	CP 5.1.2/1
Report	Validation of Analytical Method L0372/02 for the determination of BAS 750 F (Reg.No.5834378), BAS 500 F (Reg.No.304428) and BAS 700 F (Reg.No.5094351) in bee-related matrices by HPLC-MS/MS, Obermann, M., 2020 report No 878352 2020/2000481 Authority registration No
Guideline(s):	EPA 850.6100, SANCO/3029/99 (11 July 2000), SANCO/825/00 rev. 8.1 (16/11/2010)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany)
Acceptability:	Yes

Please note that the method was validated for fluxapyroxad, mefentrifluconazole, and pyraclostrobin. Only results for fluxapyroxad are presented in the summary below as this is the only compound relevant for the intended use.

Materials and methods

The analytical method L0372/02 was validated for the determination of fluxapyroxad (BAS 700 F, Reg.No. 5094351) in bee-related matrices (flowers, nectar surrogate and pollen) by LC-MS/MS with a limit of quantification (LOQ) of 0.01 mg/kg.
An amount of 0.2 g of sample material is transferred into a culture tube and extracted with methanol/water (75/25, v/v). This extraction is done twice for flower and pollen samples. The combined extracts are filled up with methanol/water (75/25, v/v). Aliquots are cleaned-up using a QuEChERS dSPE kit. After clean-up an aliquot is further diluted with methanol/water (75/25, v/v) prior to final determination by LC-MS/MS. Detection is performed in ESI positive mode, monitoring mass transition 382 m/z → 362 m/z for quantification and 382 m/z → 342 m/z for confirmation. Separation is achieved by using a Phenomenex Synergi Fusion-RP 80A column (50 mm x 2 mm, 4 µm) applying a gradient mixture of water containing 0.1% formic acid and methanol containing 0.1% formic acid at a flow rate of 0.4 mL/min.

Results and discussions

The method was confirmed to be suitable to determine fluxapyroxad in bee-related matrices. Samples were spiked with the analyte at LOQ and 10x LOQ. All average recovery values (mean of five replicates per fortification level and matrix) were between 70% and 110% and relative standard deviations (RSD) were < 20%. The detailed results are given in the table below.

Table A 1: Recovery results from method validation of fluxapyroxad using analytical method L0372/02

Analyte	Matrix	Mass Transition	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Fluxapyroxad	Flowers	382 → 362	0.01	5	99.6	3.2	101	4.2
			0.1	5	102	5.1		
		382 → 342	0.01	5	101	3.7	102	4.3
			0.1	5	103	5.0		
	Nectar surrogate	382 → 362	0.01	5	97.3	2.5	98.5	2.3
			0.1	5	99.7	1.3		
		382 → 342	0.01	5	98.6	3.2	98.7	2.5
			0.1	5	98.7	1.9		
	Pollen	382 → 362	0.01	5	109	14	107	9.6
			0.1	5	104	1.7		
		382 → 342	0.01	5	109	15	108	10
			0.1	5	106	1.9		

RSD = Relative standard deviation

Table A 2: Characteristics for the analytical method used for validation of fluxapyroxad residues in bee-related matrices

	fluxapyroxad
Specificity	The method L0372/02 determines residues of fluxapyroxad in bee-related matrices (flowers, nectar surrogate and pollen) using LC-MS/MS for final determination. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered. LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of fluxapyroxad in bee-related matrices
Calibration (type, number of data points)	Linearity of detector response was tested using six calibration standard concentrations in the range of 0.02 ng/mL to 1.0 ng/mL with correlation coefficients of ≥ 0.998. Matrix-matched calibration standards were used for the quantification of fluxapyroxad in flower and pollen samples and solvent standards, prepared in methanol/water (75/25, v/v), for nectar surrogate samples. The LOQ falls within the calibration range determined
Calibration range	Calibration points distributed over a concentration range of 0.02 ng/mL to 1.0 ng/mL were used. This corresponds to 0.0025 mg/kg – 0.13 mg/kg at test sample level.
Assessment of matrix effects is presented	Yes. Solvent- and matrix-matched standards were analyzed to assess potential matrix effects. Significant matrix effects (i.e > ±20% signal suppression or signal enhancement) were detected in flower and pollen samples, but not in nectar surrogate samples. Therefore, matrix matched standards were used for the quantification of fluxapyroxad in flower and pollen samples and solvent standards for nectar surrogate samples.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg, corresponding to the lowest fortification level at test sample level and 0.08 ng/mL at measurement sample level. The limit of detection (LOD) is 0.0025 mg/kg, corresponding to the lowest calibration standard of 0.02 ng/mL.
Stability	Stability was confirmed for fluxapyroxad in stock, fortification and calibration solutions for a maximum duration of 28 days, when stored at approximately +4 °C in the dark. Stock- and fortification solutions were prepared in methanol, and calibration solutions were prepared in methanol/water (75/25, v/v). The experiments demonstrate that fluxapyroxad was stable in extracts and final sample volumes, both prepared in methanol/water (75/25, v/v), over a time period of 7 days (8 days for nectar surrogate extracts and 10 days for nectar surrogate final volumes), when stored at approximately +4°C in the dark.

Conclusion

The method L0372/02 for analysis of fluxapyroxad in bee-related matrices (flowers, nectar surrogate and pollen) using LC-MS/MS for final determination, which is a highly specific technique, fulfils the requirements with regard to linearity, specificity, repeatability, LOQ and recoveries with a LOQ of 0.01 mg/kg.

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)

Comments of zRMS:	Evaluated at EU level

Reference:	CP 5.2/1
Report	Independent laboratory validation of BASF analytical method L0143/01: Validation of analytical method L0143/01: Determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 in water by HPLC/MS-MS, Perez, R., Perez, S., 2011 report No SubNo-201101-10-02,US-2K10-ADPEN-903-0817A,US-395759 2011/7001254 Authority registration No
Guideline(s):	EPA 164-2, EPA 835.6200, EPA 835.7100, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by United States Environmental Protection Agency)
Acceptability:	Yes

An LC-MS/MS method for the determination of fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 in water with a limit of quantification (LOQ) of 0.03 µg/L was validated in an independent laboratory.

Materials and methods

Residues are extracted from homogenized water samples (50 ml) acidified with 0.3 ml formic acid by a Phenomex Strara X-AW SPE column conditioned with 5 ml methanol and 1% formic acid in water. After clean up the column is washed with 5 ml of 1% formic acid in water and vacuum dried. Thereafter the column is eluted with 10 ml 91:10 methanol:formic acid. The extract is evaporated and reconstituted in 1.5 ml methanol:DI water. Prior to HPLC-MS/MS analysis. Analysis is conducted using a Waters Atlantis T3 column (3 µm) with a methanol:water gradient and 0.1% formic acid as modifier at a flow rate of 0.5 mL/min. Detection is accomplished in a positive and negative ionization modes monitoring mass transitions 382→362 and 382→342 for fluxapyroxad, 175→91 and 175→111 for M700F001, 161→141 and 161→97 for M700F002 and 176→156 and 176→136 for M700F007 for quantification and confirmation, respectively.

Results and discussions

The results of the recovery experiments indicate that the independent laboratory validation was successfully completed. The results show that the method is suitable to determine fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 in water. Samples spiked with the analytes at the limit of quantification of 0.03 µg/L and ten times higher (0.30 µg/L) yielded average recovery values (mean of five replicates per fortification level and analyte) between 70% and 110% and relative standard deviations (RSD) of < 20 %, except for confirmatory transition of M70F001 fortified at 0.03 µg/L with 24.2% RSD. Detailed results are summarized in the table below.

Table A 3: Recovery results from independent validation of fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	BAS 700 F	0.03 (n = 5)	91	11.6	m/z 382→362
		0.3 (n = 5)	102	13.3	m/z 382→362
		0.03 (n = 5)	82	10.9	m/z 382→342
		0.3 (n = 5)	99	9.4	m/z 382→342
	M700F001	0.03 (n = 5)	85	13.2	m/z 175→91
		0.3 (n = 5)	99	10.7	m/z 175→91
		0.03 (n = 5)	102	24.2	m/z 175→111
		0.3 (n = 5)	96	6.5	m/z 175→111
	M700F002	0.03 (n = 5)	100	8.4	m/z 161→141
		0.3 (n = 5)	93	6.0	m/z 161→141
		0.03 (n = 5)	96	18.3	m/z 161→97
		0.3 (n = 5)	103	7.6	m/z 161→97
	M700F007	0.03 (n = 5)	75	5.7	m/z 176→156
		0.3 (n = 5)	85	3.3	m/z 176→156

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		0.03 (n = 5)	74	5.4	m/z 176→136
		0.3 (n = 5)	82	3.7	m/z 176→136

Table A 4: Characteristics for the analytical method used for validation of fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 in water

	BAS 700 F, M700F001, M700F002 and M700F007
Specificity	LC-MS/MS, using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required. No residues of fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 above 30% of the LOQ were found in any of the untreated water samples.
Calibration	A seven-point standard curve was prepared by injecting standards to bracket every 2-5 samples.
Calibration range	Standards in the range of 0.0002-0.050 ng were injected and the response was plotted against the concentration. Linear correlations with coefficients ≥0.990 were obtained for fluxapyroxad and its metabolites for both mass transitions.
Assessment of matrix effects is presented	No
Limit of determination/quantification	The limit of quantification (LOQ) defined by the lowest successfully tested fortification level was 0.03 µg/L for fluxapyroxad and its metabolites M700F001, M700F002 and M700F007. The limit of determination (LOD) here defined as 30% of the LOQ was set to 0.006 µg/L.

Conclusion

The results of the independent laboratory validation confirm the results of the validation study reported in BASF DocID 2009/1069396.
The method L0143/01 is suitable for the determination of fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 in water using LC-MS/MS. It could be demonstrated that the method fulfills the requirements with regards to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to determine residues of fluxapyroxad and its metabolites M700F001, M700F002 and M700F007 in water.

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:	Evaluated at EU level

Reference:	CP 5.2/2
Report	Validation of BASF analytical method L0352/01 for the determination of BAS 700 F (Fluxapyroxad) in body fluids Richter, S., Djedovic, S., 2016 report No EU-819457,P 4055 G 2016/1217548 Authority registration No
Guideline(s):	EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Materials and methods

The analytical method is derived from the QuEChERS multi-residue method. Homogenized specimens are extracted with acetonitrile. After addition of $MgSO_4$, NaCl and buffering citrate salts, the mixture is shaken intensively and centrifuged. For urine samples, an extract aliquot is diluted prior to LC-MS/MS analysis. For blood samples, an aliquot of the organic extract is cleaned-up by addition of PSA and $MgSO_4$. After shaking and centrifugation, an extract aliquot is diluted followed by LC-MS/MS analysis. Analysis was accomplished using a Thermo Betasil column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analyzed at mass transition 382 → 362 for quantitation and 382 → 342 for confirmation for fluxapyroxad.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in both matrices tested. The relative standard deviations (RSD, %) for both commodities and fortification levels were <10%. Method validation data are summarized in the table below.

Table A 5: Recovery results from method validation of fluxapyroxad using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Urine	BAS 700 F	0.01 (n=5)	93.9	4.5	Quantitation
		0.10 (n=5)	96.6	1.2	m/z 382→362
		0.01 (n=5)	94.0	5.0	Confirmation
		0.10 (n=5)	95.7	0.8	m/z 382→342
Blood	BAS 700 F	0.01 (n=5)	103	1.4	Quantitation
		0.10 (n=5)	105	0.7	m/z 382→362
		0.01 (n=5)	103	1.6	Confirmation
		0.10 (n=5)	105	0.8	m/z 382→342

Table A 6: Characteristics for the analytical method used for validation of fluxapyroxad in body fluids

	Fluxapyroxad
Specificity	The method L0352/01 determines residues of fluxapyroxad in body fluids. The interferences/residues of the analyte measured in the control samples were below 20% of the LOQ for each matrix and each mass transition.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile / water (20/80, v/v). At least seven calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained.
Calibration range	Calibration points distributed over a concentration range of 0.01 to 1.0 ng/mL were used.
Assessment of matrix effects is presented	No significant matrix effects (i.e. > 20% suppression or enhancement) on LC-MS/MS response were observed for the matrices. The calibration standards in solvent were used for the evaluation of the results.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.01 mg/kg. The limit of detection (LOD) of the method was defined as the lowest analyte concentration injected as a calibration solution, resulting in an LOD of 0.002 mg/kg (20% of the LOQ).

	Fluxapyroxad
Stability	BAS 700 F indicated sufficient stability (<13% difference) in stock / spike (both methanol) for 30 days as well as in calibration solutions (acetonitrile/water 20/80, v/v + 0.1% formic acid; <10% difference) in final volume when stored refrigerated in the dark. Final sample extracts in acetonitrile/water (20/80, v/v) + 0.1% formic acid were re-injected after 8 days of storage under refrigerated conditions. No significant decrease (80.1-85.1% of initial value) in recovery in the stored final extracts was observed when the results were evaluated with freshly prepared calibration solutions in solvent. Thus stability of final extracts is considered sufficiently proven for at least 8 days under refrigerated storage conditions. When selected raw extracts in acetonitrile were re-analyzed after 8 days of storage under refrigerated conditions no significant decrease in recovery in the stored raw extracts was observed (80.1-84.5% of initial value).

Conclusion

The method uses highly specific LC-MS/MS for final determination of fluxapyroxad with a limit of quantitation of 0.01 mg/kg. Thereby, it could be demonstrated that the method fulfils the requirements with regards to specificity, linearity, repeatability, limit of quantitation and recoveries.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for azoxystrobin

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

For new or additional studies see A 2.2.2.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.2.2.2.1 Analytical method 1

Comments of zRMS:	Evaluated in RR of PPP Amistar Gold by zRMS-DE The method is considered reliable for the determination of residues of azoxystrobin in milk, eggs, muscle, fat, liver and kidney with a limit of quantification of 0.01 mg/kg.

Reference: CP 5.2
Report: Azoxystrobin and R230310: Validation of analytical method RAM 339/01 for the determination of residues in bovine muscle, fat and milk, lamb's kidney and liver and hen's eggs, Richards, S., 2002
report no. RJ3350B, MET2003-158
Guideline(s): Not stated
Deviations: No
GLP: Yes
Acceptability: Acceptable

Materials and methods

Residues of azoxystrobin are extracted from sample material by macerating with acetonitrile. After centrifugation the extracts are cleaned up by SPE on a C18 material. Elution is performed with ethyl acetate/dichloromethane (55/45, v/v). The eluate is evaporated to dryness and dissolved in acetonitrile/water (1/1, v/v). Final determination is done by LC-MS/MS using a RP18 column. One MS/MS transitions are monitored after electrospray ionization in positive mode: m/z 404→372.

Results and discussions

Table A 7: Recovery results from method validation of meat, milk, eggs, fat, liver, kidney using the analytical method. Standards were prepared in acetonitrile/water (1/1, v/v).

Matrix	Fortification level (mg/kg)	No of samples per fortifications level	Mean recovery	RSD (%)	Comments
Meat	0.01	5	98	2.2	m/z 404 → 372
Meat	0.1	5	96	1.6	
Fat	0.01	5	97	2.7	
Fat	0.1	5	96	2.4	
Milk	0.01	5	94	4.8	
Milk	0.1	5	94	2.2	
Kidney	0.01	5	99	4.5	
Kidney	0.1	5	97	3.1	
Liver	0.01	5	91	4.5	
Liver	0.1	5	95	2.1	
Egg	0.01	5	95	2.9	
Egg	0.1	5	96	3.0	

Table A 8: Characteristics for the analytical method used for the quantitation of azoxystrobin residues in meat, milk, eggs, fat, liver, kidney

	Azoxystrobin, m/z 404 → 372
Calibration function	Intercept forced to zero. y = 1E+08x, R^2 =0.9996 With intercept: y = 1E+08x + 3559.7, R^2 =0.9996
Accepted calibration range in concentration units (e.g. in µg/ml or ng/µl)	0.05 – 10 ng/mL
Corresponding calibration range in mass ratio units for the sample (e.g.in mg/kg or µg/L)	0.005 – 1 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)? (yes/ no)	Yes
Assessment of matrix effects is presented (yes/no)	Yes
Interference >30% of LOQ in blank sample is absent (yes/no)	Yes

Conclusion

The method of Richards, 2002 is validated for the quantification of azoxystrobin in milk, meat, fat, eggs, liver and kidney. The validated limit of quantification is 0.01 mg/kg. No significant matrix effects (>20%) were detected. A validated confirmatory method is not included.

A 2.2.2.2.2 Independent laboratory validation

Comments of zRMS:	Evaluated in RR of PPP Amistar Gold by zRMS-DE The method is considered reliable for the determination of residues of azoxystrobin in milk and muscle with a limit of quantification of 0.01 mg/kg.

Reference:	CP 5.2
Report	Independent laboratory validation of a method for the determination of residues of Azoxystrobin in animal tissue Atkinson, 2003 report no. CEMR-1907, MET2003-159
Guideline(s):	Yes; SANCO/825/00 rev. 6
Deviations:	No
GLP:	Yes
Acceptability:	Acceptable

Materials and methods

The study validated the analytical method of Richards, 2002 in an independent laboratory. The extraction and clean-up is identically with the method of Richards, 2002. Final determination is done by LC-MS/MS using a RP18 column using 5 – 10 times less diluted final extract. One MS/MS transition is monitored after electrospray ionization in positive mode: m/z 404→372.

Results and discussions

Table A 9: Recovery results from the independent laboratory validation of meat and milk using the analytical method. Standards were prepared in acetonitrile/water (1/1, v/v).

Matrix	Fortification level (mg/kg)	No of samples per fortifications level	Mean recovery	RSD (%)	Comments
Meat	0.01	5	95	2.5	m/z 404 → 372
Meat	0.1	5	101	8.7	
Milk	0.01	5	94	2.3	
Milk	0.1	5	97	6.4	

Table A 10: Characteristics for the analytical method used for the independent laboratory validation of azoxystrobin residues in milk and meat

	Azoxystrobin, m/z 404 → 372
Calibration function	y = 4085038x + 225, R^2 =0.9991
Accepted calibration range in concentration units (e.g. in µg/ml or ng/µl)	0.05 – 10 ng/mL
Corresponding calibration range in mass ratio units for the sample (e.g.in mg/kg or µg/L)	0.0005 – 0.2 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)? (yes/ no)	Yes
Assessment of matrix effects is presented (yes/no)	Yes
Interference >30% of LOQ in blank sample is absent (yes/no)	Yes

Conclusion

The method of Richards, 2002 is successfully validated in an independent laboratory for meat and milk. The validated limit of quantification is 0.01 mg/kg. A confirmatory method is not included.

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.2.2.4.1 Confirmatory method

Comments of zRMS:	Evaluated in RR of PPP Amistar Gold by zRMS-DE The method is considered reliable for the determination of residues of azoxystrobin in drinking and surface water with a limit of quantification of 0.05 µg/L.

Reference:	CP 5.2
Report	Azoxystrobin; Azoxystrobin - Validation of analytical method for the determination of Azoxystrobin and its metabolite R234886 in water - Validation report, Amic, S., 2012 report No: S11-03538, ASB2014-7343
Guideline(s):	Yes, SANCO/3029/99 Rev 4, SANCO/825/00 Rev 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues were determined after direct injection by high performance liquid chromatography with using a Kromasil KR100 5C18 column and triple quadrupole mass spectrometric detection (LC-MS/MS) in positive ionization mode with m/z 404→372 and m/z 404→344. Results and discussions

Table A 11: Recovery results from method validation of drinking and surface water using the analytical method. Standards were prepared in solvent (drinking water) and matrix (surface water).

Matrix	Fortification level (mg/kg)	No of samples per fortifications level	Mean recovery	RSD (%)	Comments
Drinking water	0.05	5	101	3	m/z 404 → 372
Drinking water	0.5	5	98	6	
Surface water	0.05	5	94	6	
Surface water	0.5	5	101	6	
Drinking water	0.05	5	104	7	m/z 404 → 344
Drinking water	0.5	5	102	8	
Surface water	0.05	5	95	14	
Surface water	0.5	5	102	4	

Table A 12: Characteristics for the analytical method used for the quantitation of azoxystrobin residues in drinking and surface water

	Azoxystrobin (drinking water)	Azoxystrobin (surface water)
Calibration function	y = 5629x – 513 R^2 =0.9963	y = 5992x – 477 R^2 =0.9945
Accepted calibration range in concentration units (e.g. in µg/ml or ng/µl)	0.0125 – 2.55 µg/L	0.0125 – 2.55 µg/L
Corresponding calibration range in mass ratio units for the sample (e.g.in mg/kg or µg/L)	0.0125 – 2.55 µg/L	0.0125 – 2.55 µg/L
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)? (yes/ no)	Yes (8 levels)	Yes (8 levels)
Assessment of matrix effects is presented (yes/no)	Yes	Yes
Interference >30% of LOQ in blank sample is absent (yes/no)	No	No

Conclusion

The method of Amic, 2012, is suitable according to the number of level (2) and the fortified samples per level (5), the recovery, the repeatability, the selectivity (blank value) and the calibration. It should be noted that analysis is done by direct injection. Hence, the calculation of recoveries is strictly speaking not possible since no extraction step is included. Water samples are characterized (pH, silt content, DOC, hardness). The method was tested for matrix effects. Matrix effects were below -10%. The study includes validation data for the metabolite R234886. Validation data for the metabolite R234886 are not reported here.

A 2.2.2.4.2 Independent Laboratory Validation (ILV)

Comments of zRMS:	The ILV of method for the determination of residues of azoxystrobin in water is accepted

Reference:	CP 5.2/6
Report	Azoxystrobin - Independent laboratory validation of analytical method GRM057.01A for the determination of residues of azoxystrobin and its metabolite R234886 in water; Brown, D.; 2019; Report RES-00193, ASB2019-16220
Guideline(s):	OECD ENV/JM/MONO(2007)17 EPA OCSPP 850.6100 EPA OCSPP 850.1340 SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Residues of azoxystrobin are determined after direct injection into LC-MS/MS. Drinking water and surface water samples were characterized. Final determination is done by LC-MS/MS using a RP18 column. Two MS/MS transitions are monitored after electrospray ionization in positive mode: m/z 404→372, 404→344.

Results and discussions

Table A 13: Recovery results from independent laboratory validation of azoxystrobin using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)
Drinking water	Azoxystrobin m/z 404 → 372	0.05 (5) 0.5 (5)	102 100	0.6 1.0
Surface water	Azoxystrobin m/z 404 → 372	0.05 (5) 0.5 (5)	101 96	1.0 1.1
Drinking water	Azoxystrobin m/z 404 → 344	0.05 (5) 0.5 (5)	102 101	1.2 0.8
Surface water	Azoxystrobin m/z 404 → 344	0.05 (5) 0.5 (5)	101 96	1.4 0.9

Table A 14: Characteristics for the analytical method used for independent laboratory validation of azoxystrobin residues in water

	Azoxystrobin m/z 404→372	**Azoxystrobin m/z 404→344**
Specificity	Mass spectrum is provided blank value <30% of LOQ	Mass spectrum is provided blank value <30% of LOQ
Calibration (type, numbers of data points)	Individual calibration data presented calibration line equation presented number of datapoints: 8	Individual calibration data presented calibration line equation presented number of datapoints: 8
Calibration range	Accepted calibration range in concentration units: 0.0125-2.5 ng/mL Corresponding calibration range in mass ratio units for the samples: 0.0125-2.5 µg/L	Accepted calibration range in concentration units: 0.0125-2.5 ng/mL Corresponding calibration range in mass ratio units for the samples: 0.0125-2.5 µg/L
Assessment of matrix effects in presented	Yes	Yes
Limit of determination/quantification	0.05 µg/L	0.05 µg/L

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

For the description of the new method for the analysis in body tissues see chapter A 2.2.2.2.1 (analytical method for the analysis in animal matrices).

A 2.2.2.7 Other Studies/ Information

New methods that are not EU peer-reviewed are published in Registration Report of the Product: AMISTAR GOLD (A18253A) to Germany due to the national assessment by the applicant Syngenta Agro GmbH (30/07/2014 Submission date) in July 2018 (dRR Syngenta July 2018).