FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CHR/H/CFF 250 EC

Product name(s): Hapi 250 EC/ Turango 250 EC

Chemical active substance(s):

Clopyralid, 120 g/L
Fluroxypyr-acid, 120 g/L (as fluroxypyr-meptyl, 172.9 g/L)
Florasulam, 10 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: March 2023

Update: November 2023

Finalisation date: January 2024; November 2024

Version history

When	What
November 2023	Update of dRR by Applicant
January 2024	zRMS assessment of dRR
November 2024	The final Registration Report

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5 Analytical methods

In the following document, data for active substances - Fluroxypyr - was described during its inclusion on Annex 1 process in respectively 2009. Where reference to active substance data in the current risk assessment has been made, it was based on the data which protection for expired 10 years from date of inclusion of active substances on Annex I.
Data matching studies for florasulam have been evaluated by Poland. As a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the authorization of CHR/H/CFF 250 EC INNVIGO is allowed to refer to EU approved reports
Data matching studies for clopyralid have been evaluated by RMS - Finland. As a result of the assessment all re-ports were accepted and considered as equivalent to protected studies. Therefore, to support the renewal of authorization of CHR/H/CFF 250 EC INNVIGO is allowed to refer to EU approved reports.

5.1 Conclusion and summary of assessment

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:

- None

Noticed data gaps are:
• Fluroxypyr:
- ILV method for drinking water,
- methods for the analysis of body fluids and tissues.
Noticed data gaps should be addressed at renewal of the CHR/H/CFF 250 EC.

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)

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 Clopyralid, Fluroxypyr and Florasulam in plant protection product is provided as follows:

Comments of zRMS:	The proposed analytical method is suitable for the simultaneous determination of active substances florasulam, fluroxypyr, and copyralid in plant protection product TURANGO 250 EC. The proposed analytical method has been fully validated in terms of specificity, linearity, repeatability, and recovery. The proposed method fulfils the requirements of SANCO/3030/99 rev.5 guidance. The validation of the analytical method has been accepted.

Reference:	KCP 5.1.1/01
Report	Validation of analytical method for CHR/H/CFF 250 EC for determination of clopyralid, clopyralid and fluroxypyr and impurities 2,6-difluoroaniline and 1-methyl-2-pyrrolidinone, Final Report Part 1, I. Knapik, 2023, Study code: ICB/45/2022
Guideline(s):	SANCO/3030/99 rev.5 22/03/19
Deviations:	No
GLP:	Yes
Acceptability:	yes

Materials and methods

Validation was carried out according to Standard Operational Procedure SPB/179. Content of clopyralid at a level of 120 g/L, florasulam at a level of 10 g/L and fluroxypyr at a level of 120 g/L in the test item was determined accordingly by liquid chromatography with diode array detection (HPLC-DAD).

Equipment and materials.
- acetonitrile HPLC (VWR),
- 85% phosphoric acid (Merck),
- placebo,
- clopyralid standard; Sigma Aldrich; batch BCBZ5263,
- florasulam standard; Sigma Aldrich; batch BCCC5799,
- fluroxypyr 1-methylheptyl ester standard; Sigma Aldrich; batch BCCF6585,
- standard stock solution of clopyralid in acetonitrile (for calibration) (Table 2),
- standard stock solution of florasulam in acetonitrile (for calibration) (Table 3),
- standard stock solution of fluroxypyr 1-methylheptyl ester in acetonitrile, the concentration of which was converted to fluroxypyr, (for calibration) (Table 4),
- working standard solutions of clopyralid, florasulam and fluroxypyr in acetonitrile (for calibration) (Table 5),
- standard stock solution of clopyralid in acetonitrile (for recovery) (Table 6),
- standard stock solution of florasulam in acetonitrile (for recovery) (Table 7),
- standard stock solution of fluroxypyr 1-methylheptyl ester in acetonitrile, the concentration of which was converted to fluroxypyr, (for recovery) (Table 8),
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland), WP/16,
- liquid chromatograph with diode array detection (Shimadzu, Japan), WP/19, WP/42, for clopyralid 245 nm, for florasulam, for fluroxypyr
- primary chromatographic system – chromatography column type C18, 100Å, 150 mm x 4,6 mm, 5 µm (Kinetex, Phenomenex), K/12/HPLC, mobile phase: acetonitrile : 0.1% H3PO4
- secondary chromatographic system –chromatography columns type C18, 100Å, 150 mm x 4,6 mm, 5 µm; Luna Omega Polar, Phenomenex, K/15/HPLC, mobile phase: acetonitrile : 0.1% H3PO4
- chromatographic vials 1.5 mL with septa buthyl/Teflon,
- volumetric flask A class 10 mL,
- measuring syringes 10 µl, 50 µL ,100 µL, 250 µL, 500 µL, 1000 µL.

Validation - Results and discussions

Specificity of the method was evaluated based on the analysis of chromatograms for placebo and samples against chromatograms of standards clopyralid, florasulam, fluroxypyr. Analysis showed no overlapping of determined ingredients signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled.

Results for primary chromatographic system.

Active ingredient (linearity range)	Linearity (n=5)
clopyralid (1.072 – 107.2 µg/mL)	R^2= 0.9999709
florasulam (1.054 – 10.54 µg/mL)	R^2= 0.9996542
fluroxypyr (1.057 – 105.7 µg/mL)	R^2= 0.9998751

Validation level	Active ingredient	Precision [%] (n=5)	Horwitz ratio	Recovery (marginal) [%] (n=5)	Standard addition [%]
100% without standard addition	clopyralid	0.19	0.10	-	-
100% with standard addition (20-30%)	clopyralid	-	-	97.31-99.75 (mean: 98.32, RSD=0.94%)	25.68
LOQ	clopyralid	2.35	0.70	100.9 (average)	-
ULOQ	clopyralid	0.87	0.35	98.6 (average)	-
100% without standard addition	florasulam	0.30	0.11	-	-
100% with standard addition (20-30%)	florasulam	-	-	97.91-106.05 (mean: 100.75, RSD=3.3%)	26.81
LOQ	florasulam	2.15	0.63	100.3 (average)	-
ULOQ	florasulam	0.96	0.39	98.9 (average)	-
100% without standard addition	fluroxypyr	0.26	0.14	-	-
100% with standard addition (20-30%)	fluroxypyr	-	-	99.48-102.45 (mean: 100.83, RSD=1.09%)	25.43
LOQ	fluroxypyr	1.76	0.52	102.9 (average)	-
ULOQ	fluroxypyr	1.02	0.41	102.8 (average)	-

Results for secondary chromatographic system.

Active ingredient (linearity range)	Linearity (n=5)
clopyralid (1.072 – 107.2 µg/mL)	R^2= 0.9999069
florasulam (1.054 – 10.54 µg/mL)	R^2= 0.9998654
fluroxypyr (1.057 – 105.7 µg/mL)	R^2= 0.9998322

Validation level	Active ingredient	Precision [%] (n=5)	Horwitz ratio	Recovery (marginal) [%] (n=5)	Standard addition [%]
100% without standard addition	clopyralid	0.34	0.18	-	-
100% with standard addition (20-30%)	clopyralid	-	-	97.03-98.44 (mean: 97.48, RSD=0.57%)	25.54
LOQ	clopyralid	1.05	0.31	99.8 (average)	-
ULOQ	clopyralid	0.63	0.26	98.2 (average)	-
100% without standard addition	florasulam	0.29	0.11	-	-
100% with standard addition (20-30%)	florasulam	-	-	93.94-103.59 (mean: 99.68, RSD=3.69%)	26.61
LOQ	florasulam	1.37	0.40	103.2 (average)	-
ULOQ	florasulam	0.87	0.35	98.7 (average)	-
100% without standard addition	fluroxypyr	0.25	0.13	-	-
100% with standard addition (20-30%)	fluroxypyr	-	-	99.17-101.12 (mean: 99.88, RSD=0.74%)	25.54
LOQ	fluroxypyr	1.20	0.36	94.7 (average)	-
ULOQ	fluroxypyr	1.04	0.42	101.9 (average)	-

Conclusion

It was confirmed that the method is specific. There were no peaks from placebo interfering with determined compounds. The validation parameters (specificity, linearity, instrument precision, repeatability, accuracy and LOQ) are within the acceptance range and fulfil EU requirements given in SANCO /3030 /99 rev.5.

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

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

Comments of zRMS:	The analytical methods for the determination of relevant impurities (difluoroaniline and 1-methyl-2-pyrrolidinone) in plant protection product TURANGO 250 EC are suitable for the determination of the content of each of the relevant impurity in the presence of each other, active substance and other components. The methods have been fully validated. The validation parameters of proposed analytical methods – interference, specificity, linearity, recovery, repeatability, and LOQ values fulfil the requirements of SANCO/3030/99 rev. 5 guidance. The validation of the analytical methods has been accepted.

Reference:	KCP 5.1.1/02
Report	Validation of analytical method for CHR/H/CFF 250 EC for determination of clopyralid, ~~clopyralid~~ florasulam and fluroxypyr and impurities 2,6-difluoroaniline and 1-methyl-2-pyrrolidinone, Final Report Part 2, I. Knapik, 2023, Study code: ICB/45/2022
Guideline(s):	SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

2,6-difluoroaniline

Validation was carried out according to Standard Operational Procedure SPB/179. Content of 2,6-difluoroaniline in the test item was determined by liquid chromatography with diode array detection (HPLC-DAD).

Equipment and materials.

- acetonitrile (Merck),
- 85% phosphoric acid (VWR),
- placebo,
- 2,6-difluoroaniline standard; Sigma Aldrich; batch STBK7160, prod. date: April 7, 2022; exp. date: April 7, 2024; storage conditions: 4±2°C,
- 2,6-difluoroaniline standard stock solution in acetonitrile (Table 3),
- working standard solution of 2,6-difluoroaniline in acetonitrile (Table 4),
- working standard solutions of 2,6-difluoroaniline for calibration in acetonitrile (Table 5),
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland), WP/16,
- liquid chromatographs with diode array detection (Shimadzu, Japan), WP/19, WP/42, 225 nm
- chromatography columns type C18 (250 mm x 4,6 mm, 5 µm); Agilent Zorbax Eclipse Plus, K/11/HPLC; K/10/HPLC; mobile phase: acetonitrile : 0.1% H_3PO_4
- chromatographic vials 1,5 mL with septa buthyl/teflon,
- volumetric flasks class A, 10 mL,
- measuring syringes 100 µL, 250 µL, 500 µl and 1000 µL.

Validation - Results and discussions

Specificity of the method was evaluated based on the analysis of chromatograms for placebo and samples against chromatograms of standard 2,6-difluoroaniline. Analysis showed no overlapping of determined impurity signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled.
Linearity range (n=5): 0.2118 – 0.8472 µg/mL
Recovery (placebo with standard addition): n=5
Precision: n=5

Results for primary chromatographic system.

Impurity	Linearity
2,6-difluoroaniline	R^2= 0.9988188

Validation level	Impurity	Precision [%]	Horwitz ratio	Recovery [%]
100%	2,6-difluoroaniline	1.76	0.26	97.1 (average)
LOQ	2,6-difluoroaniline	6.93	0.92	92.6 (average)

Results for secondary chromatographic system.

Impurity	Linearity
2,6-difluoroaniline	R^2= 0.9999073

Validation level	Impurity	Precision [%]	Horwitz ratio	Recovery [%]
100%	2,6-difluoroaniline	0.50	0.07	101.2 (average)
LOQ	2,6-difluoroaniline	1.30	0.17	103.6 (average)

1-methyl-2-pyrrolidinone

Validation was carried out according to Standard Operational Procedure SPB/179. Content of 1-methyl-2-pyrrolidinone in the test item was determined by gas chromatography with mass detection (GC- MS).

Equipment and materials.

- gas chromatograph Shimadzu GC2010 with mass detection (GC-MS), WP/38 (Shimadzu, Japan),
- chromatography column ZB-624 30 m, I.D. = 0.25 mm, df = 1.4 µm,K/4/GC (Phenomenex),

GC		**MS**	
Injection Temp	220°C	Acquisition Mode	SCAN
Pressure	52.5 kPa	Scan Range	35,0 – 500,0 m/z
Total Flow	14.0 mL/min	Event Time	0.3 sec
Septum Purge Flow	3.0 mL/min	Interface Temp	230°C
Injection Mode	Split	Sovent Cut Time	5.0 min
Split ratio	1:10	Detector Voltage	Relative to tuning result +0.2 kV
Column Flow	1.0 mL/min	Threshold	100
Carrier Gas	Hel	Ion Source Temp.	200°C

Program Temp		
Rate [ºC/min]	Temp [ºC]	Hold [min]
-	40	2
6	200	0
25	250	40

Settings SIM		
Compound name	**Target ions**	**Reference ions**
Fluorobenzene	96	70, 50
1-methyl-2-pyrrolidinone	99	98, 71

Validation - Results and discussions

Specificity of the method was evaluated based on the analysis of chromatograms for placebo and samples against chromatograms of standard 1-methyl-2-pyrrolidinone. In placebo 1-methyl-2-pyrrolidinone was detected. Specificity and selectivity of validated method for 1-methyl-2-pyrrolidinone determination was assessed at the point of optimizing conditions of analysis, by obtaining parameters for the best impurity separation while maintaining interference impact at its lowest. 1-methyl-2-pyrrolidinone was identified by the presence of specific fragmentation ions and determined by the target ions. The method was set up in the way the percentage ratio between value of the main ion to identified reference ions doesn't exceed 30% value of the error from the mass spectrum. Ions have been selected by NIST 11 library.
Calibration curve of 1-methyl-2-pyrrolidinone was prepared to determine the linear range. Linearity range (n=5): 0.333 – 11.100 µg/mL
To determine the average content of 1-methyl-2-pyrrolidinone in placebo and precision, series of measurements (n=5) of the placebo without standard addition was prepared.
To determine the average content of 1-methyl-2-pyrrolidinone (validation level 100%) and precision, the series of measurements (n=5) of the test item without standard addition was prepared.
The determine recovery, the series of measurements (n=5) of the test item with the addition of standard was prepared.
The average recovery and precision for validation level LOQ was determined by preparing series of measurements (n=5) of solvent (acetone) with standard addition.

Impurity	Linearity
1-methyl-2-pyrrolidinone	R^2= 0.998742

Validation level	Impurity	Precision [%]	Horwitz ratio	Recovery [%]	Standard addition [%]
Placebo	1-methyl-2-pyrrolidinone	2.29	0.33	-	-
100% without standard addition	1-methyl-2-pyrrolidinone	2.14	0.37	-	-
100% with standard addition (20-30%)	1-methyl-2-pyrrolidinone	-	-	95.19-113.84	29.76
LOQ	1-methyl-2-pyrrolidinone	5.10	0.73	105.3 (average)	-

Conclusion

It was confirmed that the method is specific. The validation parameters (specificity, linearity, instrument precision, repeatability, accuracy and LOQ) are within the acceptance range and fulfil EU requirements given in guidance.

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

Please refer to PART C – Confidential data.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Analytical methods for determination of penoxsulam impurities and relevance of CIPAC methods in ~~CHR/H/PENDIF 599.5 SC~~ CHR/H/CFF 250 EC were not evaluated as part of the EU review. Therefore,

all relevant data are provided and are considered adequate.

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 ~~penoxsulam, diflufenican and flufenacet~~ clopyralid, fluroxypyr and florasulam for the generation of pre-authorization data is given in the following table.

Table 5.2-1: Validated methods for the generation of pre-authorization data

Component of residue definition: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"				
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	Primary	0.01 mg/kg	LC–MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Component of residue definition: Clopyralid and its salts				
Animal products, food of animal origin	Primary	0.01 mg/kg	LC-MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Component of residue definition: Clopyralid				
Surface water Drinking water	Primary	0.05 µg/L	LC -MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Soil	Primary	0.5 µg/kg	LC -MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Air	Primary	4.5 µg/m³	LC-MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
Body fluids and tissues	Primary	0.05 mg/L (urine, blood)	LC-MS/MS	EFSA Journal 2018;16(7):5389
	Confirmatory (if required)	N/A	N/A	N/A
High water, High Oil and Dry commodities	Primary	0.01 mg/kg	LC–MS/MS	P. Schlewitz, Study code: R C2135
	Confirmatory (if required)	N/A	N/A	N/A

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: diflufenican				
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,...	Primary	0.01 mg/kg		Bacher,R.. (2002)
	Confirmatory (if required)	Not required		
Animal products, food of animal origin,...	Primary	0.01 mg/kg	GC-MS	Klumpp M, 2002
	Confirmatory (if required)	Not required		
Soil	Primary	0.002 mg/kg	GC-MS	Doran A.M., McGuire G.M., 2002
	Confirmatory (if required)	Not required		
Water	Primary	0.05 mg/kg	LC-MS/MS	Bacher R. (2002)
	Confirmatory (if required)			
Air	Primary	0.04 □g/m^3	GC-MSD	Bacher R,. 2002
	Confirmatory (if required)			
Body fluids,	Primary	Not required. The active ingredient is not classified as toxic or highly toxic.		
	Confirmatory (if required)			

Component of residue definition: Florasulam				
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,... (Residues)	Primary	0.01 mg/kg	LC/MS/MS	Rodrigues Junior, A. (2011) B.5.2.1.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis Bacher, R.. (2011) B.5.2.1.1b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Foodstaff of animal origin (Residues)	Primary	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.2.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis

Component of residue definition: Florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Robaugh David A.. (2012) B.5.2.2.1b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis Lindner, M. (2011) B.5.2.2.1c, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Soil (Environmental fate)	Primary	0.05 µg/kg	LC/MS/MS	Bacher, R.. (2011) B.5.3.1.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Water (Surface Water, Ground Water and Drinking Water)	Primary	0.05 mg/L	LC/MS/MS	Class, T.. (2011) B.5.3.2.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis Souza, N.. (2011) B.5.3.2.1b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Air (Exposure)	Primary	1.5 µg/m^3	LC/MS/MS	Class, T (2011) B.5.3.3.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Body fluids and tissue (Exposure)	Primary	0.05 mg/L	LC/MS/MS	Class, T., Gocer, M. (2011) B.5.4.2a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Soil, water (Ecotoxicology)	Primary	All data was evaluted during Annex I inclusion , and no new studies are necessery. All methods are described separatly in RAR Vol3 B8 Ecotoxicology 2013. Please refer to the DAR 2013. No general analytical methods were developed for risk assessment apart those reported as specific in studies in support of ecotoxicological studies.		
	Confirmatory (if required)			

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

Component of residue definition: Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.				
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,... (Residues)	Primary	0.01 mg/kg	GC-MS	Olberding E.L., 1996 McKellar R.L.; MacGregory, A. Markley, B.J.; 1996
	Confirmatory (if required)	Not required		
Foodstaff of animal origin (Residues)	Primary	0.01 mg/kg	LC/MS/MS GC-MS	Olberding E.L., Huskin MA., 1996 Reed D.E.; Bottoms S.N., 2003 Shackelford, D.D., 2009 Senciuc, M.; Class, T., 2009
	Confirmatory (if required)	Not required		
Soil (Environmental fate)	Primary	0.01 mg/kg	GC-MS	Shackelford, D.D.;1999
	Confirmatory (if required)	Not required		
Water (Surface Water, Ground Water and Drinking Water)	Primary	5 µg/L	GC-MS	Shackelford, D.D., 2000
	Confirmatory (if required)	Not required		
Air (Exposure)	Primary	<24 µg/m3	LC/MS/MS	Bacher, R., 2009
	Confirmatory (if required)	Not required		
High water content and Dry commodities (Residues)	Primary	0.01 mg/kg	LC-MS/MS	P. Schlewitz, Study code: R C2177
	Confirmatory (if required)	Not required		

Table 5.2-4: Validated methods for the generation of pre-authorization data

Component of residue definition: CHR/H/CFF 250 EC containing clopyralid, fluroxypyr and florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology for aquatic species and non target plants)	SPE method	0.001 mg/L for clopyralid 0.0005 mg/L for florasulam	HPLC-DAD	E. Nierzędska, Study code: W-02-20, Appendix No. 4
	LLE method	0.001 mg/L for fluroxypyr-mepthyl		
	Dilution method	0.5 mg/L for clopyralid and fluroxypyr-mepthyl 0.05 mg/L for florasulam		
	Confirmatory (if required)	Not required		
Elendt M7 (Ecotoxicology for aquatic species)	Primary	0.001 mg/L for clopyralid and fluroxypyr-mepthyl 0.0005 mg/L for florasulam	HPLC-DAD	Z. Kaceperek-Karetta, Study code: W-03-20, Appendix No. 4
	Confirmatory (if required)	Not required		
Soil (Ecotoxicology for soils organisms)	Primary	1.0 mg/kg for clopyralid and fluroxypyr-methyl 0.1 mg/kg for florasulam	HPLC-DAD	P. Pieczka, Study code: G-01-20, Appendix No. 2
	Confirmatory (if required)	Not required		
Sucrose solution (Ecotoxicology for bees)	Primary	5.0 mg/kg for clopyralid and fluroxypyr-methyl 0.5 mg/kg for florasulam	HPLC-DAD	E. Kulec-Płoszczyca, Study code: B-18-20, Appendix No. 5
	Confirmatory (if required)	Not required		
Deionized water (Ecotoxicology for bees)	Primary	Florasulam: 0.182 mg/L Clopyralid: 2.166 mg/L Fluroxypyr-meptyl: 3.146	LC/MS/MS	M. Świstak, Study code: 0038/0065/FA

Component of residue definition: CHR/H/CFF 250 EC containing clopyralid, fluroxypyr and florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
		mg/L		
	Confirmatory (if required)	Not required		

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

Data provided on Annex I inclusion is sufficient for post-authorizations methods. All data is de-scribed in EU approved documents for :

- RAR, Clopyralid - Volume 3, Annex B.5: Methods of analysis
- DAR, Diflufenican - Volume 3, Annex B.5: Methods of analysis
- DAR, Flufenacet - Volume 3, Annex B.5: Methods of analysis

Methods are described and presented in Table 5.2-3 in point KCP 5.1.2.

5.1.1 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.

5.1.2 Description of analytical methods for the determination of residues of clopyralid (KCP 5.2)

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

Table 5.2-5: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Component of residue definition: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat forage (wet crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		

Lettuce (wet crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Wheat grain (dry crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012; RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Rye grain (dry crops)	Primary	0.01 mg/kg	LC–MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Orange (acidic crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012; RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Lemon (acidic crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013),RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Canola seed (oily crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 Vogl, E. 2012; RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		

Oilseed Rape Seed((oily crops)	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 130729(2013), RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2020, S19-00446*
	Confirmatory (if required)	N/A		
Component of residue definition: Clopyralid and its salts				
Milk	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*EFSA Journal 2018;16(7):5389 120483(2012), 120484(2012),; 130729(2013)Volume3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		
Eggs	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 (2012)120483; 120484; 130729(2013)RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 120483(2012); 120484(2013); 130729(2013)RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A		
Liver	Primary	0.01 mg/kg	LC-MS/MS	*EFSA Journal 2018;16(7):5389 (2012)120483; 130729(2013) RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*
	Confirmatory (if required)	N/A	N/A	N/A
Fat	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*EFSA Journal 2018;16(7):5389 (2012)120483; 130729(2013) RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00447*

	Confirmatory (if required)	N/A		
Component of residue definition: Clopyralid and its salts				
Surface water Drinking water	Primary	0.05 µg/L	LC -MS/MS	*EFSA Journal 2018;16(7):5389 Shaffer, S. (2012); RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2019, S19-00449*
	Confirmatory (if required)	N/A		
Soil	Primary	0.5 µg/kg	LC -MS/MS	*EFSA Journal 2018;16(7):5389 Vincent, T.P. (2013); RAR, Volume 3, Annex B-Clopyralid to which is equivalent Knop, 2019, S19-00448*
	Confirmatory (if required)	N/A		
Air	Primary	4.5 µg/m³	LC-MS/MS	*EFSA Journal 2018;16(7):5389 Bacher, R. (2012);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Kirchherr, 2019, S19-00451*
	Confirmatory (if required)	N/A		
Body fluids and tissues	Primary	0.05mg/L (urine, blood)	LC-MS/MS	*EFSA Journal 2018;16(7):5389 130727(2014);RAR, Volume 3, Annex B-Clopyralid to which is equivalent Abe, 2019, S19-00450*
	Confirmatory (if required)	N/A		

5.1.2.2 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 Clopyralid and its metabolites in plant matrices is given in the following tables.

Table 5.2-6: 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: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"				
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 wet crop	Primary	Wheat forage 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,2020, S19-00446(tomato)*
		Lettuce 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR, Volume 3, Annex B-Clopyralid which is equivalent to Knop M.,(2020), S19-00446(tomato)*
	ILV	Lettuce 0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014) RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richer S., (2020), S19-00438(tomato)*
		Wheat Whole Plant	LC-MS/MS	*Austin, R. (2012), RAR, Volume 3, Annex B-Clopyralid Clopyralid to which is equivalent to Richer S., (2020), S19-00438(tomato)*
	Confirmatory (if required)	N/A		
High protein/high starch content (dry)	Primary	Wheat grain 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(rice)*
		Rye grain 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR, Volume 3, Annex B-Clopyralid which is equivalent to Knop M.,(2020), S19-00446 (rice)*
	ILV	0.01 mg/kg	LC-MS/MS	*Austin, R. (2012), RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richer S.,(2020), S19-00438 (rice)*
	Confirmatory (if required)	N/A		
(High oil content) Oily crop	Primary	Canola seed 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(olive)*
	ILV	Oilseed Rape Seed 0.01 mg/kg	LC-MS/MS	*Austin, R. (2012), RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richer S., (2020), S19-00438(olive)*
	Confirmatory (if required)	N/A		

Component of residue definition: clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"				
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) acid crop	Primary	Orange 0.01 mg/kg	LC-MS/MS	*Vogl, E. 2012;RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(grape)*
		Lemon 0.01 mg/kg	LC-MS/MS	*130729(2013),RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop M.,(2020), S19-00446(grape)*
	ILV	Lemon 0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014) RAR, Volume 3, Annex B-Clopyralid*
	Confirmatory (if required)	N/A		

Table 5.2-7: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	RAR clopyralid Vol. 3 Section B.5
Not required, because:	Extraction efficiency requirement was described in the guideline SANTE 2017/10632 rev. 3, which was noted in November 2017, thus it is applicable only to submissions after 11.2019. EFSA for clopyralid was published in 2018, thus before the guideline enter into force.

5.1.2.3 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 clopyralid in animal matrices is given in the following tables.

Table 5.2-8: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Clopyralid and its salts				
Milk	Primary	Bovine milk 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013),RAR,Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	Bovine milk 0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014); RAR,Volume3,Annex B-Clopyralid*
	Primary	Bovine milk 0.01 mg/kg	LC-MS/MS	*120483(2012),RAR,Volume3, Annex B-Clopyralid to which is equivalent to abe Abe,Ch.(2019) S19-00447*
	ILV	Bovine milk 0.01 mg/kg	LC-MS/MS	*120484(2012) RAR,Volume3, Annex B-Clopyralid*
	Confirmatory (if required)	N/A		
Eggs	Primary	Poultry eggs 0.01 mg/kg	LC-MS/MS	*120483(2012), RAR Volume3, Annex B-Clopyralid Clopyralid to which is equivalent to Abe,Ch.(2019)S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012);), RAR Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019)P 5210 G*
	Primary	Poultry eggs 0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013),RAR,Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	Confirmatory (if required)	N/A		
Muscle	Primary	0.01 mg/kg	LC-MS/MS	*120483 (2012); RAR Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019)P 5210 G*

	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019)P 5210 G*
	Confirmatory (if required)	N/A	N/A	*N/A*
Liver, kidney	Primary	0.01 mg/kg	LC-MS/MS	*120483(2012); RAR Volume3, Annex B-Clopyralid o which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012); RAR,Volume3, Annex B-Clopyralid*
	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013);RAR Volume3, Annex B-Clopyralid to which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	N/A		
	Confirmatory (if required)	N/A		
Fat	Primary	0.01 mg/kg	LC-MS/MS	*120483(2012); RAR Volume3, Annex B-Clopyralid o which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	LC-MS/MS	*120484(2012); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019);P 5210 G*
	Primary	0.01 mg/kg	QuEChERS LC-MS/MS	*130729(2013); RAR Volume3, Annex B-Clopyralid o which is equivalent to Abe,Ch.(2019) S19-00447*
	ILV	0.01 mg/kg	QuEChERS LC-MS/MS	*130728(2014); RAR,Volume3, Annex B-Clopyralid to which is equivalent to Schweizer, M.,(2019);P 5210 G*
	Confirmatory (if required)	N/A		

Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	RAR clopyralid Vol. 3 Section B.5
Not required, because:	Extraction efficiency requirement was described in the guideline SANTE 2017/10632 rev. 3, which was noted in November 2017, thus it is applicable only to submissions after 11.2019. EFSA for clopyralid was published in 2018, thus before the guideline enters into force.

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

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

Table 5.2-5: Validated methods for soil (if appropriate)

Component of residue definition: Clopyralid				
Soil	Primary	0.5 µg/kg	LC -MS/MS	*Vincent, T.P. (2013); RAR, Volume 3, Annex B-Clopyralid to which is equivalent to* Knop, M.,(2019) *S19-00448*
	ILV	Not required.		
	Confirmatory (if required)	N/A		

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

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

Table 5.2-6: Validated methods for water (if appropriate)

Component of residue definition: Clopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water Surface water Ground water	Primary	0.05 µg/L	LC-MS/MS A validation for drinking water was not necessary because the limit of quantitation for surface water is below the drinking water limit of 0.1 µg/L.	*Shaffer, S. (2012); RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Knop, M.,(2019); S19-00449*
	ILV	0.05µg/L	LC-MS/MS	*Austin, R., Turner, R. (2013) RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Richter, S. (2019); P 5211 G*
	Confirmatory	N/A		

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

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in air is given in the following tables. For the detailed evaluation of additional studies please refer to 0.

Table 5.2-7: Validated methods for air (if appropriate)

Component of residue definition: Clopyralid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	4.5 µg/m^3	LC-MS/MS	*Bacher, R. (2012);RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Kirchher, M.(2019); S19-00451*
ILV	N/A		
Confirmatory	N/A		

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

Table 5.2-9: Validated methods for body fluids and tissues (if appropriate)

Component of residue definition: Clopyralid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	human blood, human urine 0.05 mg/L	LC-MS/MS	*130727 (2014), RAR, Volume 3, Annex B-Clopyralid to which is equivalent to Abe Ch.(2019); S19-00450*
ILV	N/A		
Confirmatory	N/A		

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5.1.2.8 Other studies/ information

Not required

5.1.3 Description of analytical methods for the determination of residues of fluroxypyr (KCP 5.2)

5.1.3.1 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 not identical.

Table 5.2-10: 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	Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.	0.01 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Plant, high acid content		0.01 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Plant, high oil content		0.01	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Muscle	Fluroxypyr	0.02 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Milk		0.01 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Eggs		0.02 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Fat		0.02 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Liver, kidney		0.02 mg/kg	~~Reg. (EU) 2017/623~~ Reg. (EU) 2022/1363
Soil (Ecotoxicology)	Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.	0.11 mg/kg	AOEL
Drinking water (Human toxicology)	Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.	0.015 mg/L	lowest NOEC [EFSA Scientific Report (2007) 122]
Air	Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.	<24 µg/m^3	AOEL sys/AOEL inhal: 0.017 mg/kg bw/d

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Tissue (meat or liver)	Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.	~~Not required~~	notclassified as T / T+
Body fluids		~~Not required~~	notclassified as T / T+

5.1.3.2 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 fluroxypyr in plant matrices is given in the following tables.

Table 5.2-11: 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: Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.				
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	0.01 mg/kg	GC-MS	Olberding E.L., 1996
	ILV	0.01 mg/kg	GC-MSD	McKellar R.L.; MacGregory, A. Markley, B.J.; 1996
	Confirmatory (if required)			

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to 0.

zRMS:
Proposed uses (cereals) belong to high protein/high starch content (dry) group commodities.
Adequate methods are available to monitor residues of Fluroxypyr in food and feed of plant origin in representatives of dry/high starch content matrixes.

Table 5.2-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	DAR Fluroxypyr, Volume 3, Annex B.5
Not required, because:	

5.1.3.3 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 fluroxypyr in animal matrices is given in the following tables.

Table 5.2-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	GC-MS	Olberding E.L., Huskin MA., 1996
	ILV	0.01 mg/kg	GC-MS	Reed D.E.; Bottoms S.N., 2003
	Confirmatory (if required)	Not required		
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Shackelford, D.D., 2009
	ILV	0.01 mg/kg	LC-MS/MS	Senciuc, M.; Class, T., 2009
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	GC-MS	Olberding E.L., Huskin MA., 1996
	ILV	0.01 mg/kg	GC-MS	Reed D.E.; Bottoms S.N., 2003
	Confirmatory (if required)	Not required		
Fat	Primary	0.01 mg/kg	GC-MS	Olberding E.L., Huskin MA., 1996
	ILV	0.01 mg/kg	GC-MS	Reed D.E.; Bottoms S.N., 2003
	Confirmatory (if required)	Not required		
Kidney, liver	Primary	0.01 mg/kg	GC-MS	Olberding E.L., Huskin MA., 1996
	ILV	0.01 mg/kg	GC-MS	Reed D.E.; Bottoms S.N., 2003
	Confirmatory (if required)	Not required		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to 0.

Table 5.2-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	DAR Fluroxypyr, Volume 3, Annex B.5
Not required, because:	-

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

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

Table 5.2-15: Validated methods for soil (if appropriate)

Component of residue definition: Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	GC-MS	Shackelford, D.D.;1999
Confirmatory	Not required		

For any special comments or remarkable points concerning the analytical methods for soil please refer to 0.

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

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

Table 5.2-16: Validated methods for water (if appropriate)

Component of residue definition: Fluroxypyr and its ester Fluroxypyr 1-methylheptylester expressed as Fluroxypyr.				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	5 µg/L	GC-MS	Shackelford, D.D., 2000
	ILV	-		
	Confirmatory	-		
Surface water	Primary	5 µg/L	GC-MS	Shackelford, D.D., 2000
	Confirmatory			

For any special comments or remarkable points concerning the analytical methods for water please refer to 0.

zRMS comment:
Data gap:
- ILV Method (for drinking water) is required by the REGULATION (EU) No 284/2013 and SANTE/2020/12830, Rev.1. This requirement may be supplemented when the product is reassessed.

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

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

Table 5.2-17: Validated methods for air (if appropriate)

Component of residue definition: Fluroxypyr acid and Fluroxypyr-MHE			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	<24 µg/m^3	LC-MS-MS	Bacher, R., 2009
Confirmatory			

For any special comments or remarkable points concerning the analytical methods for air it is referred to 0.

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

~~Not required. The active substant is not classification like as toxic or very toxic.~~

zRMS comment:
Data gap:
- Methods are required by the REGULATION (EU) No 284/2013 SANTE/2020/12830, Rev.1. This requirement may be supplemented when the product is reassessed.

5.1.3.8 Other studies/ information

Not required

5.1.4 Description of analytical methods for the determination of residues of florasulam (KCP 5.2)

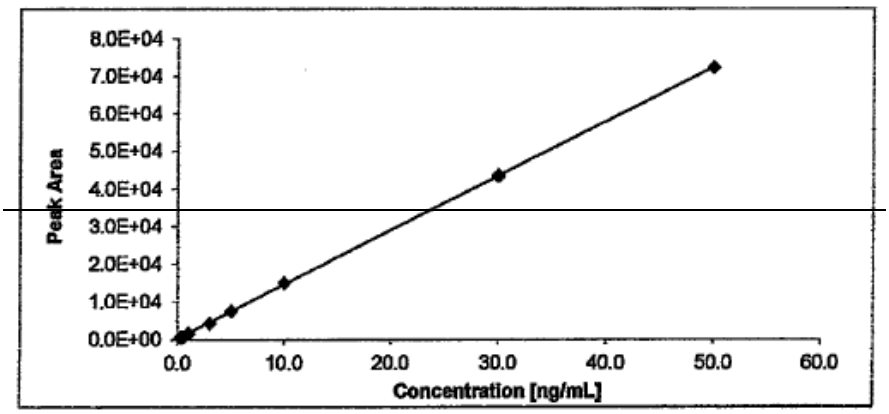
~~Reference:~~	~~KCP 5.2~~
~~Report~~	~~Final Report Determination of residues of iodosulfuron-methyl, tribenuron-methyl, florasulam and mefenpyr-diethyl after one application of IDS 100 OD or FLOT 150 WG and Adjuwant Super in wheat at 4 sites in Northern Europe 2016, J. Semrau, EAS Study Code S16-02449,~~
~~Guideline(s):~~	~~SANCO /3029 /99 rev.4.~~
~~Deviations:~~	~~NO~~
~~GLP:~~	~~YES~~
~~Acceptability:~~	~~YES~~

Materials and methods

The analytical methods multi-residue QuEChERS for the determination of residues of florasulam in wheat (whole plant, grain and straw) was validated according to SANCO/3029/99, rev. 4 within this analytical phase of this study. Quantification was performed by use of LC-MS/MS detection. The limit of quantification (LOQ) of the analytical methods was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at each 0.003 mg/kg (30 % of the LOQ). No residues above 30% of the LOQ were detected in the control (untreated) test portions used for recovery determination. All mean recovery values at fortifica-tion levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ) comply with the standard acceptance criteria of the guidance document SANCO/3029/99 rev 4, with the evaluation od two mass transitions.

Validation - Results and discussions

Table 5.2-18: Methods suitable for the determination of the residues in plant protection product (PPP) CHR/H/FLO 100 SC

	Residues
Author(s), year	J.Semrau, 2016
Principle of method	LC-MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of matrix matched and solvent calibration standards at a minimum of five concentration levels ranging from o.30 ng/ml to 100 ng/ml for determination of all analytes in wheat (whole plant) and for the determination of florasulam in wheat (grain). This range corresponds to a fortification level of 0.003 mg/kg to 1.0 mg/kg and thus covers the range from no more than 30% od the LOQ and at least +20% of the highest analyte concentration detected in any (diluted) specimen extract. The calibration curves obtained for both mass transitions for each analyte were linear with coefficients of determination (R^2) ≥ 0.980. Linear regression was performed without any weighting. Representative linear regression curve(s) are below. 
Quantification	Quantification was performed using a calibration curve that fulfilled the above given criteria. The injection of standard solutions was spread evenly over the whole analytical sequence. The average response factor was used for calculation of the analyte concentrations. The relative standard deviation of the average response factor was lower or equal to 20 %. If necessary, specimen extracts and extracts from high level recovery samples were diluted with solvent to be within the calibration range. Diluted sample extracts (at least by a factor of 10) were quantified using solvent calibration standards instead of matrix-matched calibration standards.

<table>
<tr><td></td><td>Residues</td></tr>
<tr><td>Selectivity</td><td>The analytes were determined in the final specimen extracts by use of LC-MS/MS detection.
For each analyte, one (1) mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of specimens. Untreated samples for accompanying control sample work up, for determination of (procedural) recoveries and, if needed, for preparation of matrix matched standards originated from the current study. At least one (1) control sample per each matrix type and analytical set was analysed to investigate the residue level of the analytes and to check for any background interferences at the expected retention times of the analytes.
Correction for blank values was not performed.</td></tr>
<tr><td>Matrix Effects</td><td>The effect of wheat (whole, plant, grain and straw) on the LC-MS/MS response was assessed by comparing peak areas of matrix matched standards with solvent standards at identical concentrations. During validation of the methods following matrix effects were determined:

<table>
<tr><th rowspan="2">Matrix / Commodity</th><th rowspan="2">Standard Concentration (ng/mL)</th><th colspan="2">Matrix Effect for Florasulam (%)</th></tr>
<tr><th>Quantification (358 →167 m/z)</th><th>Confirmation (358 →152 m/z)</th></tr>
<tr><td>Wheat (whole plant)</td><td>1 – 50</td><td>(+) 4.6</td><td>(+) 6.1</td></tr>
<tr><td>Wheat (grain)</td><td>1 – 50</td><td>(+) 2.1</td><td>(+) 1.7</td></tr>
<tr><td>Wheat (straw)</td><td>1 – 50</td><td>(-) 7.4</td><td>(-) 3.8</td></tr>
</table>

Matrix effects were < 20 % for Florasulam in wheat (whole plant, grain and straw), the matrix effect were deemed insignificant. Therefore solvent standards were used for quantification.

Matrix effects were once again tested during the analysis of the field samples to determine the actually conditions of mass spectrometer system. Matrix effects were < 20 % for Florasulam and in wheat (whole plant) and thus deemed to be insignificant. However, matrix-matched standards were used for quantification of field samples.</td></tr>
<tr><td>LOQ</td><td>The limit of quantification (LOQ) was 0.01 mg/kg with a limit of detection (LOD) of 0.003 mg/kg</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO/3029/99 rev.4.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANCO /3029 /99 rev.4. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

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

Compared to the residue definition proposed in the Renewal Assessment Report (incl. its addenda) the

current legal residue definition is identical.

Table 5.2-19: 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	Florasualm	LOQ 0.01 mg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Plant, high acid content		LOQ 0.01 mg/kg	
Plant, high protein/high starch content (dry commodities)		LOQ 0.01 mg/kg	
Plant, high oil content		LOQ 0.01 mg/kg	
Muscle	Florasulam	LOQ 0.01 mg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Milk		LOQ 0.01 mg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Eggs		LOQ 0.01 mg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Fat		LOQ 0.01 mg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Liver, kidney		LOQ 0.01 mg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Soil (Ecotoxicology)	Florasulam, 5-OH Florasulam	0.05 µg/kg	RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Drinking water (Human toxicology)	Florasulam, 5-OH Florasulam	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Florasulan, 5-OH Florasulam	1.18 µg a.s./L	EFSA Journal 2015; 13(1): 3984
Air	Florasulam	1.5 µg/m^3	AOEL sys/AOEL inhal: 0.05 mg/kg bw/d
Tissue (meat or liver)	Florasulam	LOQ 0.01 mg/kg	notclassified as T / T+
Body fluids		0.05 mg/L	notclassified as T / T+

5.1.4.2 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 Florasulam in plant matrices is given in the following tables.

Table 5.2-20: 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: Florasulam				
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	Rodrigues Junior, A. (2011) B.5.2.1.1.a RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Bacher R. (2011) B.5.2.1.1b RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
High acid content	Primary	0.01 mg/kg	LC/MS/MS	Rodrigues Junior A. (2011) B.5.2.1.1.a RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Bacher R. (2011) B.5.2.1.1b RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
High oil content	Primary	0.01 mg/kg	LC/MS/MS	Rodrigues Junior A. (2011) B.5.2.1.1.a RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.1.1b RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC/MS/MS	Rodrigues Junior A. (2011) B.5.2.1.1.a RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Bacher R. (2011) B.5.2.1.1b RAR Florasulam - Volume 3 Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Difficult (if required,	Primary	Not required		
	ILV			

Component of residue definition: Florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
depends on intended use)	Confirmatory (if required)			

Table 5.2-21: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Residues below LOQ

5.1.4.3 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 Florasulam in animal matrices is given in the following tables.

Table 5.2-22: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.2.1.a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Robaugh David A.. (2012) B.5.2.2.1.b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Eggs	Primary	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.2.1.a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Robaugh David A.. (2012) B.5.2.2.1.b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.2.1.a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis

Component of residue definition: Florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.01 mg/kg	LC/MS/MS	Robaugh David A.. (2012) B.5.2.2.1.b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Fat	Primary	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.2.1.a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Robaugh David A.. (2012) B.5.2.2.1.b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		
Kidney, liver	Primary	0.01 mg/kg	LC/MS/MS	Bacher, R. (2011) B.5.2.2.1.a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	ILV	0.01 mg/kg	LC/MS/MS	Robaugh David A.. (2012) B.5.2.2.1.b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory (if required)	Not required		

Table 5.2-23: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Residues below LOQ

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

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

Table 5.2-24: Validated methods for soil (if appropriate)

Component of residue definition: Florasulam			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 µg/kg	LC/MS/MS	Bacher, R.. (2011) B.5.3.1.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Confirmatory	Not required		

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

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

Table 5.2-25: Validated methods for water (if appropriate)

Component of residue definition: Florasulam				
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		Class, T.. (2011) B.5.3.2.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	ILV	0.05 µg/L		Souza, N.. (2011) B.5.3.2.1b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory	Not required		
Surface water	Primary	0.05 µg/L		Class, T.. (2011) B.5.3.2.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis Souza, N.. (2011) B.5.3.2.1b, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
	Confirmatory	Not required		

5.1.4.6 Description of methods for the analysis of air (KCP 5.2) Florasulam in air is given in the following tables.

Table 5.2-26: Validated methods for air (if appropriate)

Component of residue definition: Florasulam			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1.5 µg/m^3	LC/MS/MS	Class T. (2011) B.5.3.3.1a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Confirmatory	Not required		

5.1.4.7 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 Florasulam in body fluids and tissues is given in the following table.

Table 5.2-27: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Florasulam			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L	LC/MS/MS	Class, T., Gocer, M. (2011) B.5.4.2a, RAR, Florasulam - Volume 3, Annex B.5: Methods of analysis
Confirmatory	Not required		

5.1.4.8 Other studies/ information

No other studies are provided.

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 5.1.1/01	I. Knapik	2023	Validation of analytical method for CHR/H/CFF 250 EC for determination of clopyralid, clopyralid and fluroxypyr and impurities 2,6-difluoroaniline and 1-methyl-2-pyrrolidinone – Part 1 Study code: ICB/45/2022 ICB Pharma, 10 Lema Street, 43-600, Jaworzno, POLAND GLP Unpublished	N	Chemirol Sp. z o.o.
KCP 5.1.1/02	I. Knapik	2023	Validation of analytical method for CHR/H/CFF 250 EC for determination of clopyralid, clopyralid and fluroxypyr and impurities 2,6-difluoroaniline and 1-methyl-2-pyrrolidinone – Part 2 Study code: ICB/45/2022 ICB Pharma, 10 Lema Street, 43-600, Jaworzno, POLAND GLP Unpublished	N	Chemirol Sp. z o.o.
~~KCP 5.2~~ KCP 5.1.1./03	J.Semrau	2016	Final Report Determination of residues of iodosulfuron-methyl, tribenuron-methyl, florasulam and mefenpyr-diethyl after one application of IDS 100 OD or FLOT 150 WG and Adjuwant Super in wheat at 4 sites in Northern Europe 2016 EAS Study Code S16-02449 Eurofins Agroscience Services GmbH, Stade, Germany GLP yes Unpublished	N	PUH Chemirol Sp. z o.o.
KCP	P. Schlewitz	2023	Validation of the Analytical Method for the Analysis of Clopyralid (Sum of Clopyralid, its salts and	N	PUH

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
5.1.1/04			conjugates) in High water content, in High Oil content and Dry Commodities Study code: R C2135 ANADIAG, 16, rue Ampère, 67500 HAGUENAU, France GLP Unpublished		Chemirol Sp. z o.o. PROPLAN Plant Protection Company, S.L.U
KCP 5.1.1/05	P. Schlewitz	2023	Validation of the Analytical Method for the Analysis of Fluroxypyr (Sum of Fluroxypyr its salts, its esters and its conjugates, expressed as fluroxypyr) in High water content and Dry Commodities Study code: R C2177 ANADIAG, 16, rue Ampère, 67500 HAGUENAU, France GLP Unpublished	N	PUH Chemirol Sp. z o.o.
KCP 5.1.1/06	E. Kulec-Płoszczyca	2023	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test, Appendix No. 5. Development and validation analytical method of active substances Study code: B-18-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. z o.o.
KCP 5.1.1/07	E. Nierzędska	2023	CHR/H/CFF 250 EC Lemna gibba CPCC 310, Growth inhibition test, Appendix No. 4 Validation of analytical method for clopyralid, florasu-lam and fluoxypyr-meptyl and chemical analysis Study code: B-18-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. z o.o.
KCP 5.1.1/08	M. Świstak	2022	Validation of analytical method for determination of active substances florasulam, clopyralid and fluroxypyr (in the form of fluroxypyr-meptyl) of the test item CHR/H/CFF 250 EC in deionized water	N	PUH Chemirol Sp.

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
			Study code: 0038/0065/FA SORBOLAB Research Laboratory LLC, 11 Zaniemyska Street, Poznań 61-029, Poland GLP Unpublished		z o.o.
KCP 5.1.1/09	Z. Kacpere-Karetta	2023	CHR/H/CFF 250 EC Daphnia magna, Acute Immobilisation Test, Appendix No. 4 Validation of analytical method for clopyralid, florasu-lam and fluoxypyr-meptyl and chemical analysis Study code: W-03-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. z o.o.
KCP 5.1.1/10	P. Pieczka	2023	CHR/H/CFF 250 EC Earthworm reproduction test (Eisenia andrei), Appendix No. 2. Results of analytical measurements Study code: G-01-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna Ecotoxicology Research Group, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Unpublished	N	PUH Chemirol Sp. z o.o.

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 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
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KCP 5.2		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* 120483 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* 120484 GLP-yes unpublished	N	DAS

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		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2	Shaffer, S.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS 120611 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2	Vincent, T.P.	2013	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS* 120612 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2	Bacher, R.	2012	*The Development and Validation of a Method for the Analysis of Clopyralid in Air* 120601 PTRL Europe GmbH, D-89081 Ulm, Germany GLP-yes unpublished	N	DAS
KCP 5.2		2014	*Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)* 130727 GLP-yes unpublished	N	DAS

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	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2		2014	*Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130728 GLP-yes unpublished	N	DAS
KCP 5.2	Austin, R.	2012	*Independent Laboratory Validation of Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS"* 120614 Battelle UK Ltd, Ongar, Essex, United Kingdom GLP-yes unpublished	N	DAS
KCP 5.2	Vogl, E.	2012	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* 120610 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS

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		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2	Austin, R.	2012	*Independent Laboratory Validation of Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS"* 120614 Battelle UK Ltd, Ongar, Essex, United Kingdom GLP-yes unpublished	N	DAS
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KCP 5.2		2014	*Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130728 GLP-yes unpublished	N	DAS

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		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2		2014	*Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130728 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* 120483 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* 120484 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* 120483 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* 120484 GLP-yes unpublished	N	DAS

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		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
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KCP 5.2		2012	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* 120484 GLP-yes unpublished	N	DAS
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KCP 5.2		2012	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* 120483 GLP-yes unpublished	N	DAS

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		2012	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* 120484 GLP-yes unpublished	N	DAS
KCP 5.2		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2		2012	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* 120483 GLP-yes unpublished	N	DAS
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KCP 5.2		2013	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130729 GLP-yes unpublished	N	DAS
KCP 5.2		2014	*Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* 130728 GLP-yes unpublished	N	DAS

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	Vincent, T.P.	2013	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS* 120612 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2	Austin, R., Turner, R.	2013	Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS" 120613 Battelle UK Ltd, Ongar, Essex, United Kingdom GLP-yes unpublished	N	DAS
KCP 5.2	Shaffer, S.	2012	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS 120611 ABC Laboratories, Inc., Columbia, Missouri, USA GLP-yes unpublished	N	DAS
KCP 5.2	Austin, R., Turner, R.	2013	Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS" 120613 Battelle UK Ltd, Ongar, Essex, United Kingdom GLP-yes unpublished	N	DAS

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	Bacher, R.	2012	*The Development and Validation of a Method for the Analysis of Clopyralid in Air* 120601 PTRL Europe GmbH, D-89081 Ulm, Germany GLP-yes unpublished	N	DAS
KCP 5.2		2014	*Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)* 130727 GLP-yes unpublished	N	DAS
KCP 5.2	Rodrigues Junior A.	2011	*Residue Metod Validation for the Determination of Florasulam in Agricultural Commodities* *Das Report No. 110535* *Mogi Mirim Reg. Lab., Brazil* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Bacher R.	2011	*Florasulam: Independet Laboratory Validation of Residue Method for the Determination of Florasulam in Agricultural Commodities.* *DAS Report No. 110536* *PTRL EUROPE Gmbh, Ulm, Germany* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Bacher R.	2011	*Method Validation Study for the Determination of Residues of Forasulam in Foodstaff and Animal Origin bt Liquid Chromatography with Tandem Mass Spectrometry* *DAS Report No. 110540* *PTRL Europe Gmbh, Ulm, Germany* *GLP yes* *Unpublished*	N	DAS

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	Robaugh David A.	2012	*Independet Laboratory Validation Study for the determination of Residues of Florasulam in Bolvine and Poultry Tissues by Liquid Chromatography with Tandem Mass Spectrometry* *DAS Report No. 110541* *Pyxant Labs Inc., Colorado Srings, USA* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Lindner M.	2011	*Examination of the Applicability of the Modular Analytical Method L 00.00-34 for the Determination of Residues of Florasulam* *DAS Report No. 110671* *Eurofins Agrosciences Services Chem Gmbh, Hamburg, Germany* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Bacher R.	2011	*Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabolite in Soil by Liquid Chromatography with Tandem Mass Spectrometry* *DAS Report No. 110537* *PTRL Europe Gmbh, Ulm, Germany* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Class T.	2011	*Method Validation Study for the Determination of Residues of Florasulam and its 5-0h Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry* *DAS Report No. 110538* *PTRL Europe Gmbh, Ulm, Germany* *GLP yes* *Unpublished*	N	DAS

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	Souza N.	2011	*Independet Laboratory Validation of Dow AgroSciences LLC Method – Determination of Residues of Florasulam and its 5-OH Metabolite in Surface Water, Ground Water and Drinking Water ny Liquid Chromatography with Tandem Mass Spectrometry Detection* *DAS Report No. 110539* *Dow AgroSciences Ind., Mogi Mirim, Brazil* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Class T.	2011	*The Development and Validationof a Method for the Analysis of Florasulam in Air* *DAS Report No. 110282* *PTRL Europe Gmbh, Ulm, Germany* *GLP yes* *Unpublished*	N	DAS
KCP 5.2	Class T, Göcer M.	2011	Florasulam: Develpoment of an Analytical Method for the Determination of Florasulam in Body Fluid(s) DAS Report No. 110283 PTRL Europe Gmbh, Ulm, Germany GLP yes Unpublished	N	DAS

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	Olberding, E. L., Ng, C.A.	1996	Validation report for the determination of residues of Fluroxypyr and Fluroxypyr-1-methylheptyl ester as the acid equivalent in the grain, forage, straw, and hay of wheat, barley, and oats by capillary gas chromatography with mass selective detection, (GRM 96.02, supplementary) Global Environmental Chemistry Laboratory, Indianapolis, Indiana, USA 1996-06-04 including: Determination of residues of Fluroxypyr 1-methylheptyl ester as the acid equivalent in the grain, forage, straw, and hay of wheat, barley, and oats by capillary gas chromatography with mass selective detection, GRM 96.02 Wildlife International Ltd., Maryland, USA 1996-03-27 GH-C 4049 (RES 95118) GLP: yes not published	N	DOW

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	McKellar, R. L., MacGregor, J.A., Markley, B.J.	1996	Independent laboratory validation of method GRM 96.02 – Determination of Fluroxypyr and Fluroxypyr 1-Methylheptyl ester as the acid equivalent in the grain, forage, straw, and hay of wheat, barley, and oats by capillary gas chromatography with mass selective detection Wildlife International Ltd., Maryland, USA GH-C 4166 (RES96044) 1996-08-02 GLP: yes not published	N	DOW
KCP 5.2	Olberding, E.L., Huskin, M.A.	1996	Determination of Fluroxypyr in ruminant tissues and milk by capillary gas chromatography with mass selective detection Pyxant Labs Inc, Colorado GRM 96.03, Study ID 030053 Annex to report PTR No. 30198040-5008-1, ref. IIA, 4.8 /04 1996-03-27 GLP: no not published	N	DOW

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	Olberding, E.L., Huskin, M.A.	1996	Validation report for the determination of residues of Fluroxypyr in ruminant tissues and milk by capillary gas chromatography with mass selective detection (validation data for analytical method GRM 96.03) Global Environmental Chemistry Laboratory, Indianapolis, Indiana, USA GH-C 4048 1996-06-10 GLP: yes not published	N	DOW
KCP 5.2	Reed, D.E., Bottoms, S.N.	2003	Independent laboratory validation of Dow AgroSciences LLC method GRM 96.03 – Determination of residues of Fluroxypyr in ruminant tissues and milk by gas chromatography with mass selective detection Pyxant Labs Inc., Colorado Springs, USA PTR No. 30198040-5008-1 2003-11-25 GLP: yes not publied	N	DOW
KCP 5.2	Shackelford, D.D.	2009	Determination of Residues of Fluroxypyr in Poultry Tissues and Eggs by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences LLC, Indianapolis, Indiana, Lab. Study I.D. 081043 GLP Unpublished	N	DOW

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	Senciuc, M., Class, T..	2009	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 08.03 - Determination of Residues of Fluroxypyr in Poultry Tissues and Eggs by High Performance Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences Protocol No. 080153, Study No. P 1545G GLP Unpublished	N	DOW
KCP 5.2	Shackelford, D.D.	1999	Method validation report for the determination of Fluroxypyr and its major metabolites in soil by gas chromatography with mass selective detection. GRM 98.04 Global Environmental Chemistry Laboratory, Indianapolis, Indiana, USA GH-C 4908 1999-06-09 GLP: yes not published	N	DOW
KCP 5.2	Shackelford, D.D.	1999	Independent laboratory validation method GRM 98.04 – Determination of residues of Fluroxypyr and its major metabolites in soil by capillary gas chromatography with mass selective detection, GRM 98.04 Enviro-Bio-Tech Ltd., Bernville, USA GH-C 4985 1999-09-21 GLP: yes not published	N	DOW

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	Shackelford, D.D.	2000	Validation report for the determination of residues of Fluroxypyr 1-methylheptyl ester, Fluroxypyr-acid, Fluroxypyr-2-pyridinol, and Fluroxypyr-2-methoxypyridine in surface water by capillary gas chromatography with mass spectrometric detection, GRM 00.21 Global Environmental Chemistry Laboratory, Indianapolis, Indiana, USA GH-C 5157 2000-12-15 GLP: yes not published	N	DOW
KCP 5.2	Bacher, R.	2009	The Development and Validation of a Method for the Analysis of Fluroxypyr-acid and Fluroxypyr-1-methylheptyl ester in Air DOW Study No. 091018, PTRL Report No. B1644 G. GLP Unpublished	N	DOW

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**

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

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

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

Detailed evaluation of submitted analytical methods

Analytical methods for ~~penoxsulam~~ florasulam

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

No new or additional studies have been submitted

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

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

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP ~~5.2~~ 5.1.1/03
Report	Final Report Determination of residues of iodosulfuron-methyl, tribenuron-methyl, florasulam and mefenpyr-diethyl after one application of IDS 100 OD or FLOT 150 WG and Adjuwant Super in wheat at 4 sites in Northern Europe 2016, J. Semrau, EAS Study Code S16-02449,
Guideline(s):	SANCO /3029 /99 rev.4.
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods

The analytical methods multi-residue QuEChERS for the determination of residues of florasulam in wheat (whole plant, grain and straw) was validated according to SANCO/3029/99, rev. 4 within this analytical phase of this study. Quantification was performed by use of LC-MS/MS detection. The limit of quantification (LOQ) of the analytical methods was 0.01 mg/kg for each analyte and each matrix with a limit of detection (LOD) set at each 0.003 mg/kg (30 % of the LOQ). No residues above 30% of the LOQ were detected in the control (untreated) test portions used for recovery determination. All mean recovery values at fortifica-tion levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ) comply with the standard acceptance criteria of the guidance document SANCO/3029/99 rev 4, with the evaluation od two mass transitions.

Validation - Results and discussions

Table 5.2-28: Methods suitable for the determination of the residues in wheat for florasulam

	Residues
Author(s), year	J.Semrau, 2016
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of the detector response was demonstrated by single determination of matrix-matched and solvent calibration standards at a minimum of five concentration levels ranging from o.30 ng/ml to 100 ng/ml for determination of all analytes in wheat (whole plant) and for the determination of florasulam in wheat (grain). This range corresponds to a fortification level of 0.003 mg/kg to 1.0 mg/kg and thus covers the range from no more than 30% od the LOQ and at least +20% of the highest analyte concentration detected in any (diluted) specimen extract. The calibration curves obtained for both mass transitions for each analyte were linear with coefficients of determination (R^2) ≥ 0.980. Linear regression was performed without any weighting.
Quantification	Quantification was performed using a calibration curve that fulfilled the above given criteria. The injection of standard solutions was spread evenly over the whole analytical sequence. The average response factor was used for calculation of the analyte concentrations. The relative standard deviation of the average re-sponse factor was lower or equal to 20 %. If necessary, specimen extracts and extracts from high level recovery samples were diluted with solvent to be within the calibration range. Diluted sample extracts (at least by a factor of 10) were quantified using solvent calibration standards instead of matrix-matched calibration standards.
Selectivity	The analytes were determined in the final specimen extracts by use of LC MS/MS detection. For each analyte, one (1) mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantifi-cation of specimens. Untreated samples for accompanying control sample work up, for determination of (procedural) recoveries and, if needed, for preparation of matrix-matched standards originated from the current study. At least one (1) control sample per each matrix type and analytical set was analysed to investigate the residue level of the analytes and to check for any background interferences at the expected retention times of the analytes. Correction for blank values was not performed.
Matrix Effects	The effect of wheat (whole, plant, grain and straw) on the LC-MS/MS

<table>
<tr><th></th><th>Residues</th></tr>
<tr><td></td><td>response was assessed by comparing peak areas of matrix-matched standards with solvent standards at identical concentrations. During validation of the methods following matrix effects were determined:
<table>
<tr><th rowspan="2">Matrix / Commodity</th><th rowspan="2">Standard Concentration (ng/mL)</th><th colspan="2">Matrix Effect for Florasulam (%)</th></tr>
<tr><th>Quantification (358→167 m/z)</th><th>Confirmation (358→152 m/z)</th></tr>
<tr><td>Wheat (whole plant)</td><td>1 - 50</td><td>(+) 4.6</td><td>(+) 6.1</td></tr>
<tr><td>Wheat (grain)</td><td>1 - 50</td><td>(+) 2.1</td><td>(+) 1.7</td></tr>
<tr><td>Wheat (straw)</td><td>1 - 50</td><td>(-) 7.4</td><td>(-) 3.8</td></tr>
</table>
Matrix effects were < 20 % for Florasulam in wheat (whole plant, grain and straw), the matrix effect were deemed insignificant. Therefore solvent standards were used for quantification.

Matrix effects were once again tested during the analysis of the field samples to determine the actually conditions of mass spectrometer system. Matrix effects were < 20 % for Florasulam and in wheat (whole plant) and thus deemed to be insignificant. However, matrix-matched standards were used for quantification of field samples.</td></tr>
<tr><td>LOQ</td><td>The limit of quantification (LOQ) was 0.01 mg/kg with a limit of detection (LOD) of 0.003 mg/kg</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANCO/3029/99 rev.4.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANCO /3029 /99 rev.4. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

Analytical methods for ~~Diflufenican~~ Fluroxypyr

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

~~No new or additional studies have been submitted~~

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/05
Report	Validation of the Analytical Method for the Analysis of Fluroxypyr (Sum of Fluroxypyr its salts, its esters and its conjugates, expressed as fluroxypyr) in High water content and Dry Commodities, P. Schlewitz, 2023, Study Code: R C2177
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Equipment
- Usual laboratory glassware
- Centrifuge with centrifuge tube of 50 mL or other equivalent material
- Vortex
- Ultra-turrax
- Orbital shaker
- Ultrasonic bath
- Evaporation under nitrogen system (sand bath at 40 °C)
- Drying oven (at 90°C)

- pH paper

5.4.2 Solvents and reagents

Name	CAS No.	Formula	Quality
Acetone	67-64-1	C_3H_6O	for analysis
Acetonitrile	75-05-8	C_2H_3N	HPLC
Water	7732-18-5	H_2O	Type 1
Sodium hydroxide	1310-73-2	NaOH	for analysis
Hydrochloric acid 37%	7647-01-0	HCl	for analysis
Formic acid (conc.)	64-18-6	HCOOH	for analysis
Magnesium sulfate (1)	7487-88-9	$MgSO_4$	for analysis
Sodium chloride (1)	7647-14-5	NaCl	for analysis
Supel QuE Non-Buffered Tube (2)	-	4 g $MgSO_4$ + 1 g NaCl	Sigma-Aldrich ref. 55294-U
PET 0.2 µm filter	-	-	for analysis

(2) is an alternative to (1)

Residues of fluroxypyr 1-MHE and fluroxypyr were extracted from high water content and dry commodities samples by homogenizing with an acetone/0.25 M hydrochloric acid (60/40, v/v) solution. After centrifugation an aliquot of the extract is taken and fluroxypyr 1-MHE is hydrolyzed to fluroxypyr by adding a sodium hydroxide solution. Acetone is evaporated and hydrochloric acid is added. The vial is placed into a oven set at 90°C to hydrolyze releasing any fluroxypyr conjugates. After addition of acetonitrile, magnesium sulfate and sodium chloride, the raw extract is purified with a liquid-liquid partition. An aliquot of the upper layer is filtered and analysed by LC-MS/MS.

Validation - Results and discussions

Table 5.2-29: Methods suitable for the determination of the residues in wheat for fluroxypyr

	Residues
Author(s), year	P. Schlewitz, 2023
Principle of method	LC MS/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The analytical calibration consisted of matrix-matched calibration solutions of fluroxypyr, at least at 5 concentration levels, ranged from 0.4 ng/mL to 30 ng/mL (corresponding to 0.002 to 0.12 mg/kg for fluroxypyr, to 0.002 to 0.17 mg/kg for fluroxypyr-1-methylheptyl ester and to 0.003 to 0.20 mg/kg for fluroxypyr conjugated form). The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods. The calibration covered two orders of magnitude and ranged from 20% of the LOQ to 20% above the highest level for fluroxypyr, ranged from 20% of the LOQ to 70% above the highest level for fluroxypyr-1-methylheptyl ester and ranged from 30% of the LOQ to 100% above the highest level for fluroxypyr conjugates (based of fluroxypyr-glucoside). Standard concentrations were distributed evenly over the full calibration range. Calibration curves were run for each analysis sequence for both primary and confirmatory methods. The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods..
Recovery and repeat-	For samples fortified at 0.01 mg/kg, mean recoveries were within the acceptable range 60-120% with RSD less than 30% for both primary and confirmatory methods.

Residues

ability

For samples fortified at 0.10 mg/kg, mean recoveries were within the acceptable range 70-120% with RSD less than 20% for primary method.

Summary of recoveries – Primary method

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Fluroxypyr	High water content (Wheat whole plant)	0.01	105.4%	3.8%	100.3%	110.2%	5
		0.10	109.9%	2.1%	106.6%	112.2%	5
Fluroxypyr-1-methylheptyl ester		0.01	109.0%	4.9%	100.7%	114.7%	5
		0.10	113.1%	5.3%	107.0%	121.8%	5
Fluroxypyr	Dry commodities (wheat grain)	0.01	90.6%	7.5%	80.3%	96.9%	5
		0.10	96.9%	10.4%	86.2%	109.6%	5
Fluroxypyr-1-methylheptyl ester		0.01	107.4%	14.5%	86.4%	129.3%	5
		0.10	115.4%	13.1%	94.5%	129.9%	5

For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.2 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

Requirements for mean recovery and precision (SANTE/2020/12830, Rev.2)

Concentration level	Range of mean recovery (%)	Precision, RSD (%)
0.01 mg/kg	60-120	30
0.1 mg/kg	70-120	20

Summary of recoveries – Confirmatory method

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Fluroxypyr	High water content (Wheat whole plant)	0.01	101.2%	3.0%	98.6%	104.6%	5
Fluroxypyr-1-methylheptyl ester		0.01	110.2%	6.9%	99.6%	120.4%	5
Fluroxypyr	Dry commodities (wheat grain)	0.01	92.2%	7.9%	83.8%	102.1%	5
Fluroxypyr-1-methylheptyl ester		0.01	110.3%	12.0%	98.1%	132.6%	5

For the confirmatory method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.2 guideline as mean recoveries were within the range 60-120% and RSD were less than 30% for spiked samples at the LOQ level.

Selectivity

Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte/matrix combination and for both primary and confirmatory methods were provided to prove selectivity of the method.
Mass spectra were provided to justify the selection of ions used for determination.
Untreated samples (non-fortified samples) were determined from the matrices used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods.

Matrix Effects

Matrix effects, expressed in % enhancement or suppression, were assessed for each commodities and analyte, for both primary and confirmatory methods. They were considered significant if they exceeded ±20%.
Matrix effects (enhancement or suppression) on the instrument response were considered significant for most of commodities/analyte combination. Consequently, matrix-matched calibration solutions were used for calibration.

<table>
<tr><td></td><td>Residues</td></tr>
<tr><td></td><td>
Matrix effects – Primary method
<table>
<tr><th>Matrix</th><th>Analyte</th><th>Concentration (ng/mL)</th><th>Matrix effect (%)</th></tr>
<tr><td>High water content (Wheat whole plant)</td><td>Fluroxypyr</td><td>25.0</td><td>-34.9%</td></tr>
<tr><td>Dry commodities (Wheat grain)</td><td>Fluroxypyr</td><td>25.0</td><td>-28.3%</td></tr>
</table>
Matrix effects – Confirmatory method
<table>
<tr><th>Matrix</th><th>Analyte</th><th>Concentration (ng/mL)</th><th>Matrix effect (%)</th></tr>
<tr><td>High water content (Wheat whole plant)</td><td>Fluroxypyr</td><td>25.0</td><td>-37.7%</td></tr>
<tr><td>Dry commodities (Wheat grain)</td><td>Fluroxypyr</td><td>25.0</td><td>-30.2%</td></tr>
</table>
The detailed data are given in appendix II.
Matrix effects (enhancement or suppression) on the instrument response were considered significant. Consequently, matrix-matched calibration solutions were used for calibration.
</td></tr>
<tr><td>LOQ</td><td>The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision.
The LOQ was 0.01 mg/kg for fluroxypyr and fluroxypyr-1-methylheptyl ester in high water content and in dry commodities.</td></tr>
<tr><td>LOD</td><td>The limit of detection (LOD) was expressed as lowest calibration standard.
The LOD was 0.4 ng/mL for fluroxypyr in high water content and in dry commodities (corresponding to 0.002 mg/kg for fluroxypyr, to 0.002 mg/kg for fluroxypyr-1-methylheptyl ester and to 0.003 mg/kg for fluroxypyr conjugated form (based of fluroxypyr-glucoside)).</td></tr>
<tr><td>Confirmation</td><td>The confirmatory method was required to confirm that the primary method detected the correct analyte (analyte identity) and that the analyte signal of the primary method was quantitatively correct and not affected by any other compound.
Confirmation simultaneously to primary detection:
The confirmatory method was achieved by monitoring 1 additional transition for high water content and for dry commodities.
<table>
<tr><th></th><th>Primary transition</th><th>Confirmatory transition</th></tr>
<tr><td>Fluroxypyr</td><td>m/z 252.9 > 232.8</td><td>m/z 252.9 > 194.5</td></tr>
</table>
</td></tr>
<tr><td>Stability results for extracts</td><td>The stability of extracts during frozen storage was investigated.
Fluroxypyr residues were stable in extracts for at least 22 days of frozen storage for high water content and 15 days of frozen storage for dry commodities.</td></tr>
</table>

Residues

Recoveries after frozen storage of extracts

Sample ANADIAG No.	Commodities	Fortification level (mg/kg)	Storage time (days)	Fluroxypyr % Recovery Primary method 252.9 > 232.8
C2177 01 01 FA STAB	High water content (Wheat whole plant)	0.10	22	102.4%
C2177 01 01 GA STAB				105.3%
C2177 01 01 HA STAB				105.3%
C2177 01 01 IA STAB				104.9%
C2177 01 01 JA STAB				109.9%
C2177 03 01 FA STAB	Dry commodities (Wheat grain)	0.10	15	86.2%
C2177 03 01 GA STAB				108.6%
C2177 03 01 HA STAB				100.3%
C2177 03 01 IA STAB				93.3%
C2177 03 01 JA STAB				101.3%

Summary of recoveries

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Fluroxypyr	High water content (Wheat whole plant)	0.10	105.6%	2.6%	102.4%	109.9%	5
Fluroxypyr	Dry commodities (Wheat grain)	0.10	97.9%	8.7%	86.2%	108.6%	5

The stability of the analyte in the final extracts was sufficiently proven according to the SANTE/2020/12830, Rev.2 guideline, as mean recoveries in the fortified samples were within the range 70-120%, measured against freshly prepared standards.

Fluroxypyr residues were stable in extracts for at least 22 days of frozen storage for high water content (wheat whole plant) and 15 days of frozen storage for dry commodities (wheat grain).

Stability results for matrix-matched standard solutions

The stability of matrix-matched standard solutions during frozen storage was investigated. Fluroxypyr residues were stable in extracts for at least 21 days of frozen storage for high water content and 14 days of frozen storage for dry commodities.

Stability data for matrix-matched standard solutions after frozen storage

Analyte	Concentration level (ng/mL)	Storage time (days)	Solvent (matrix matched)	Difference between average response factors
Fluroxypyr	25	**21**	High water content (Wheat whole plant)	1.3%
Fluroxypyr	25	**14**	Dry commodities (Wheat grain)	1.6%

The difference between average response factors from at least 5 replicate measurements for each of the two solutions did not differ by more than 10%.
Fluroxypyr residues were stable in matrix-matched calibration solutions for at least 21 days of frozen storage for high water content (wheat whole plant) and 14 days of frozen storage for dry commodities (wheat grain).

Comment

The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

Analytical methods for ~~Flufenacet~~ Clopyralid

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

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/04
Report	Validation of the Analytical Method for the Analysis of Clopyralid (Sum of Clopyralid, its salts and conjugates) in High water content, in High Oil content and Dry Commodities, P. Schlewitz, 2023, Study Code: R C2135
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Equipment
- Usual laboratory glassware
- Centrifuge with centrifuge tube of 50 mL or other equivalent material
- Vortex
- Ultrasonic bath
- Evaporation under nitrogen system (sand bath at 40 °C)
- Water bath at 60 °C
- pH paper

5.4.2 Solvents and reagents

Name	CAS No.	Formula	Quality
Methanol	67-56-1	CH_3OH	HPLC
Acetonitrile	75-05-8	C_2H_3N	HPLC
Water	7732-18-5	H_2O	Type 1
Sulfuric acid conc.	7664-93-9	H_2SO_4	for analysis
Potassium hydroxide	1310-58-3	KOH	for analysis
Formic acid (conc.)	64-18-6	HCOOH	for analysis
Supel QuE Non-Buffered Tube	-	4 g $MgSO_4$ + 1 g NaCl	Sigma-Aldrich ref. 55294-U
Ammonium formate	540-69-2	HCO_2NH_4	for analysis
PTFE 0.45 µm filter	-	-	for analysis

Residues of clopyralid and its conjugates are extracted and hydrolysed from samples by heating at 60 °C for 3 hours with 2.5M KOH. After acidification with H_2SO_4, addition of acetonitrile, magnesium sulfate and sodium chloride, the raw extract is purified with a liquid-liquid partition. An aliquot of the upper layer is evaporated to dryness and the sample is reconstituted in 50:50, methanol/H_2O + 0.1% formic acid. Extracts are analysed by LC-MS/MS.

Validation - Results and discussions

Table 5.2-30: Methods suitable for the determination of the residues in wheat for clopyralid

	Residues
Author(s), year	P. Schlewitz, 2023
Principle of method	LC MS/MS

	Residues
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The analytical calibration consisted of matrix-matched calibration solutions of clopyralid, at least at 5 concentration levels, ranged from 0.4 ng/mL to 24.5 ng/mL (corresponding to 0.002 to 0.12 mg/kg for clopyralid and to 0.003 to 0.16 mg/kg for clopyralid glycine). The calibration covered two orders of magnitude and ranged from 20% of the LOQ to 20% above the highest level for clopyralid and ranged from 30% of the LOQ to 60% above the highest level for clopyralid glycine. Standard concentrations were distributed evenly over the full calibration range. Calibration curves were run for each analysis sequence for both primary and confirmatory methods. An example of a typical calibration plot, the equation of the calibration line, the linear correlation coefficient and the regression residuals plot is given in Appendix V for both primary and confirmatory methods. The linear correlation coefficients were > 0.990, showing good linearity with regression residuals randomly distributed for both primary and confirmatory methods.
Recovery and repeatability	For samples fortified at 0.01 mg/kg, mean recoveries were within the acceptable range 60-120% with RSD less than 30% for both primary and confirmatory methods. For samples fortified at 0.10 mg/kg, mean recoveries were within the acceptable range 70-120% with RSD less than 20% for primary method. ***Summary of recoveries – Primary method***

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Clopyralid	Oilseed rape whole plant (High water content)	0.01	84.2%	3.8%	80.5%	86.3%	3
		0.10	96.8%	1.7%	95.6%	98.7%	3
Clopyralid glycine		0.01	75.5%	6.3%	70.0%	78.3%	3
		0.10	83.6%	6.7%	79.5%	89.9%	3
Clopyralid	Sugar beets whole plant (High water content)	0.01	82.6%	6.1%	79.3%	88.4%	3
		0.10	98.3%	3.1%	95.4%	101.5%	3
Clopyralid glycine		0.01	64.0%	5.0%	60.3%	66.1%	3
		0.10	78.0%	2.8%	75.9%	80.3%	3
Clopyralid	Sugar beets leaves (High water content)	0.01	88.8%	7.7%	83.3%	96.4%	3
		0.10	102.2%	6.4%	96.3%	109.3%	3
Clopyralid glycine		0.01	61.7%	5.4%	58.3%	64.9%	3
		0.10	90.1%	6.5%	85.6%	96.7%	3
Clopyralid	Sugar beets roots (High water content)	0.01	80.5%	1.3%	79.3%	81.2%	3
		0.10	88.6%	3.3%	85.8%	91.7%	3
Clopyralid glycine		0.01	63.4%	4.4%	60.5%	66.0%	3
		0.10	72.8%	1.4%	71.8%	73.9%	3
Clopyralid	Wheat straw (Dry commodities)	0.01	88.8%	3.3%	86.2%	91.9%	3
		0.10	100.9%	8.0%	95.7%	110.2%	3
Clopyralid glycine		0.01	76.9%	3.5%	74.9%	79.9%	3
		0.10	74.1%	6.6%	69.4%	79.1%	3

For the primary method, recovery and repeatability (as precision, % RSD) data complied with the requirements of the SANTE/2020/12830, Rev.1 guideline as mean recoveries were within the range 60-120% with RSD less than 30% for spiked samples at 0.01 mg/kg and within the range 70-120% with RSD less than 20% for spiked samples at 0.10 mg/kg.

Requirements for mean recovery and precision (SANTE/2020/12830, Rev.1)

Concentration level	Range of mean recovery (%)	Precision, RSD (%)
0.01 mg/kg	60-120	30
0.1 mg/kg	70-120	20

Residues

Summary of recoveries – Confirmatory method

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Clopyralid	High water content (Wheat whole plant)	0.01	87.3%	17.5%	70.2%	106.1%	5
Clopyralid glycine		0.01	73.7%	25.5%	56.2%	105.2%	5
Clopyralid	High oil content (Oilseed rape seeds)	0.01	93.4%	4.0%	87.6%	97.7%	5
Clopyralid glycine		0.01	76.2%	4.8%	71.8%	81.4%	5
Clopyralid	Dry commodities (wheat grain)	0.01	96.7%	20.2%	72.4%	121.3%	5
Clopyralid glycine		0.01	85.3%	13.3%	78.4%	105.4%	5

Selectivity

Representative, clearly labelled chromatograms of standard at the lowest calibrated level, untreated sample and sample fortified at the lowest fortification level for each analyte/matrix combination and for both primary and confirmatory methods were provided to prove selectivity of the method.
Mass spectra were provided to justify the selection of ions used for determination.
Untreated samples (non-fortified samples) were determined from the matrices used in fortification experiments and were not higher than 30% of the LOQ for both primary and confirmatory methods..

Matrix Effects

Matrix effects, expressed in % enhancement or suppression, were assessed for each commodities and analyte, for both primary and confirmatory methods. They were considered significant if they exceeded ±20%.
Matrix effects (enhancement or suppression) on the instrument response were considered significant for most of commodities/analyte combination. Consequently, matrix-matched calibration solutions were used for calibration.

Matrix effects – Primary method

Matrix	Analyte	Concentration (ng/mL)	Matrix effect (%)
High water content (Wheat whole plant)	Clopyralid	20.0	**16.4%**
High oil content (Oilseed rape seeds)	Clopyralid	20.0	**-46.9%**
Dry commodities (Wheat grain)	Clopyralid	20.0	**21.6%**

Matrix effects – Confirmatory method

Matrix	Analyte	Concentration (ng/mL)	Matrix effect (%)
High water content (Wheat whole plant)	Clopyralid	20.0	**18.0%**
High oil content (Oilseed rape seeds)	Clopyralid	20.0	**-29.8%**
Dry commodities (Wheat grain)	Clopyralid	20.0	**17.0%**

Matrix effects (enhancement or suppression) on the instrument response were considered significant for most of commodities/analyte combination. Consequently, matrix-matched calibration solutions were used for calibration.

LOQ

The limit of quantification (LOQ) was the lowest validated level with sufficient recovery and precision
The LOQ was 0.01 mg/kg for clopyralid and clopyralid glycine in high water content, in high oil content and in dry commodities.

LOD

The limit of detection (LOD) was expressed as lowest calibration standard.
The LOD was 0.4 ng/mL for clopyralid in high water content, high oil content and dry commodities (corresponding to 0.002 mg/kg for clopyralid and 0.003 mg/kg for clopyralid glycine).

Confirmation

The confirmatory method was required to confirm that the primary method detected the correct analyte (analyte identity) and that the analyte signal of the primary method was quantitatively correct and not affected by any other compound.
Confirmation simultaneously to primary detection:
The confirmatory method was achieved by monitoring 1 additional transition for high water content

Residues

and for dry commodities.

	Primary transition	Confirmatory transition
Clopyralid	*m/z* 192.0 > 110.0	*m/z* 192.0 > 84.0

For high oil content, the confirmatory method was achieved by analysing 2 untreated samples and 5 fortified samples at the LOQ level with an alternative analytical column.

Analytical conditions	Column description	Bounding	Column phase
Primary Method	C18	Organic/inorganic hybrid silica-based ODS column	
Confirmatory Method	BEH-phenyl	Trifunctional C6 phenyl, fully endcapped, bonded to an Ethylene Bridged Hybrid (BEH) substrate.	

Stability results for extracts

The stability of extracts during refrigerated storage was investigated.
Clopyralid residues were stable in extracts for at least 15 days of refrigerated storage for high water content and dry commodities and 19 days of refrigerated storage for high oil content.

Recoveries after refrigerated storage of extracts

Sample ANADIAG No.	Commodities	Fortification level (mg/kg)	Storage time (days)	Clopyralid % Recovery Primary method 192.0 > 110.0
C2135 01 01 FA	High water content (Wheat whole plant)	0.10	15	**96.7%**
C2135 01 01 GA				**95.9%**
C2135 01 01 HA				**99.8%**
C2135 01 01 IA				**95.7%**
C2135 01 01 JA				**91.6%**
C2135 05 01 FA	High oil content (Oilseed rape seeds)	0.10	19	**92.9%**
C2135 05 01 GA				**87.5%**
C2135 05 01 HA				**91.3%**
C2135 05 01 IA				**93.0%**
C2135 05 01 JA			20	**87.6%**
C2135 06 01 ZA	Dry commodities (Wheat grain)	0.10	15	**111.2%**
C2135 06 01 AAA				**108.5%**
C2135 06 01 ABA				**102.0%**
C2135 06 01 ACA				**102.1%**
C2135 06 01 ADA				**91.5%**

Summary of recoveries

Analyte	Matrix	Fortification level (mg/kg)	Mean recoveries (%)	Relative standard deviation (RSD) (%)	Min recovery (%)	Max recovery (%)	Number of fortified samples (n)
Clopyralid	High water content (Wheat whole plant)	0.10	95.9%	3.1%	91.6%	99.8%	5
Clopyralid	High oil content (Oilseed rape seeds)	0.10	90.5%	3.0%	87.5%	93.0%	5
Clopyralid	Dry commodities (Wheat grain)	0.10	103.1%	7.4%	91.5%	111.2%	5

Stability results for matrix-

The stability of matrix-matched standard solutions during refrigerated storage was investigated.
Clopyralid residues were stable in matrix-matched calibration solutions for at least 14 days of refrigerated storage for high water content and dry commodities and 19 days of refrigerated storage

	Residues
matched standard solutions	for high oil content. **Stability data for matrix-matched standard solutions after refrigerated storage** Analyte: Clopyralid; Concentration level (ng/mL): 20; Storage time (days): 14; Solvent (matrix matched): High water content (Wheat whole plant); Difference between average response factors: 1.9% Analyte: Clopyralid; Concentration level (ng/mL): 20; Storage time (days): 19; Solvent (matrix matched): High oil content (Oilseed rape seeds); Difference between average response factors: 0.4% Analyte: Clopyralid; Concentration level (ng/mL): 20; Storage time (days): 14; Solvent (matrix matched): Dry commodities (Wheat grain); Difference between average response factors: 10.0%
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

Analytical methods for CHR/H/CFF 250 EC

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

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/06
Report	CHR/H/CFF 250 EC Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test,Appendix No. 5 Validation of analytical method for clopyralid, florasulam and fluoxypyr-meptyl and chemical analysis , E. Kulec-Płoszczyca, 2023, Study Code: B-18-20
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Equipment

Equipment	Size, Description	Manufacturer/Supplier	Standard Operating Procedure
Analytical Balance	Adventurer Pro AV 114CM	Ohaus Corporation (USA)	SOP/C/122
Balance	WPS 510/C	Radwag (Poland)	SOP/C/25
Volumetric flasks	Various volumes	Glassco (Germany)	SOP/C/12
Variable volume single-channel pipettes	Various volumes	Eppendorf AG (Germany)	
Autosampler vials with PTFE/silicone septa and screw caps	Clear glass, 2 mL	Alwsci Technologies (China)	-
Chromatograph	Prominence	Shimadzu Corp. (Japan)	SOP/C/304

Reagents and solvents

Chemical	Grade	Manufacturer/Supplier	Batch Number	Expiry date
Deionized water	HPLC grade	Łukasiewicz-IPO*	Fresh prepared before analysis	
Ortho-phosphoric acid	85% HPLC	SUPELCO	Z0721828108	31.07.2023
Acetonitrile	HPLC	Chempur	220601232	06.2024
		VWR Chemicals	22E194018	19.05.2025
		Chempur	220803052	08.2024

* The main stages of water purification: pre-treatment (mechanical filter, activated carbon), deionisation (ion exchange resin). Water prepared with SolPure-7 water deionizer [SOP/C/91]

The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The analytical methods were developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1 [1] and Standard Operating Procedure SOP/C/9. The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.
The concentration of active substances of test item in fresh (Day 0) and spent (Day 4) test item concentration (666.7 mg/kg) and control were chemically determined

Validation - Results and discussions

Table 5.2-31: Methods suitable for the determination of the residues in sucrose solution for CHR/H/CFF 250 EC

	Residues
Author(s), year	E.Kulec-Płoszczyca, 2023
Principle of method	HPLC-DAD
Linearity (linear between mg/L)	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Residues

(correlation coefficient, expressed as r)

Analyte	Working solution concentrations [mg/L]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/kg Sucrose solution]
clopyralid	0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0	0.05 – 5.0	0.5 – 50.0
florasulam	0.01, 0.02, 0.05, 0.1, 0.2 and 0.5	0.01 – 0.5	0.1 – 5.0
fluroxypyr-mepthyl	0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0	0.05 – 5.0	0.5 – 50.0
clopyralid	1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0	1.0 – 100.0	10.0 – 1000.0
florasulam	0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0	0.1 – 10.0	0.1 – 10.0
fluroxypyr-mepthyl	1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0	1.0 – 100.0	10.0 – 1000.0

The standard curves of clopyralid, florasulam and fluroxypyr-mepthyl (peak area versus quantity of the standard) are linear.
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linear was given in µg/mL equivalent to mg/L (equal to µg/mL).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.05 – 5.0	clopyralid	123231	-830.816	0.9999220
0.01 – 0.5	florasulam	72211.4	-123.271	0.9997957
0.05 – 5.0	fluroxypyr-mepthyl	110669	-903.182	0.9996382
1.0 – 100.0	clopyralid	122759	-132.833	0.9999876
0.1 – 10.0	florasulam	72890.5	-323.919	0.9999835
1.0 – 100.0	fluroxypyr-mepthyl	110480	-646.597	0.9999819

Recovery and repeatability

Precision
Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analyzed are presented in table below. The RSD is ≤ 20% per each level.
Accuracy
The accuracy of the methods is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70 – 120%.
A summary of the recovery data of control and fortified samples are presented in the table below.

Analyte	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]
clopyralid	5.0	5	102.0	0.3
	50.0	5	100.1	0.1
florasulam	0.5	5	101.6	1.6
	5.0	5	100.6	0.4
fluroxypyr-mepthyl	5.0	5	91.5	0.3
	50.0	5	99.0	0.1

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analyzed using the methods described above.

Selectivity

The analytical method specificity was estimated on the basic of the analysis of the chromatograms

<table>
<tr><td></td><td>Residues</td></tr>
<tr><td></td><td>obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the methods were demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Matrix Effects (sucrose solution)</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard preparing in solution to standard preparing in blank matrix at appropriate concentration. The matrix effect is not exceed ± 20%. The matrix effect and concentration are presented in table below.
<table>
<tr><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>clopyralid</td><td>0.5</td><td>0.0</td></tr>
<tr><td>florasulam</td><td>0.05</td><td>-0.1</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>0.5</td><td>-1.4</td></tr>
</table></td></tr>
<tr><td>LOQ</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
<table>
<tr><th>Analyte</th><th>LOQ [mg analyte /kg]</th><th>Equivalent calibration level [µg/mL]</th><th>LOD [mg analyte/kg]</th><th>Equivalent calibration level [µg/mL]</th></tr>
<tr><td>clopyralid</td><td>5.0</td><td>0.5</td><td>0.5</td><td>0.05</td></tr>
<tr><td>florasulam</td><td>0.5</td><td>0.05</td><td>0.1</td><td>0.01</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>5.0</td><td>0.5</td><td>0.5</td><td>0.05</td></tr>
</table></td></tr>
<tr><td>LOD</td><td>The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample.
<table>
<tr><th>Analyte</th><th>LOQ [mg analyte /kg]</th><th>Equivalent calibration level [µg/mL]</th><th>LOD [mg analyte/kg]</th><th>Equivalent calibration level [µg/mL]</th></tr>
<tr><td>clopyralid</td><td>5.0</td><td>0.5</td><td>0.5</td><td>0.05</td></tr>
<tr><td>florasulam</td><td>0.5</td><td>0.05</td><td>0.1</td><td>0.01</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>5.0</td><td>0.5</td><td>0.5</td><td>0.05</td></tr>
</table></td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/07
Report	CHR/H/CFF 250 EC Lemna gibba CPCC 310, Growth inhibition test, Appendix No. 4 Validation of analytical method for clopyralid, florasulam and fluoxypyr-meptyl and chemical analysis, E. Nierzędska, 2023, Study Code: W-02-20
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Equipment	Size, Description	Manufacturer/Supplier	Standard Operating Procedure
Analytical Balance	Adventurer Pro AV 114CM	Ohaus Corporation (USA)	SOP/C/122
Volumetric flasks	Various volumes	Glassco (Germany)	SOP/C/12
Variable volume single-channel pipettes	Various volumes	Eppendorf AG (Germany)	
Measuring cylinder	Various volumes	Different supliers	
Rotary vacuum evaporator with water medium bath	RV 05 basic HB 4 basic	IKA - WERKE (Germany)	SOP/C/118
Rotary vacuum evaporator with water medium bath	RV 10 digital HB 10 digital	IKA - WERKE (Germany)	SOP/C/329
Laboratory shaker	WL - 2000	J.W.Electronic (Poland)	SOP/C/16
Indicator papers	pH 1-14	ChemLand	-
Laboratory timer	-	TFA Dostmann GmbH & Co. KG	-
Autosampler vials with PTFE/silicone septa and screw caps	Clear glass, 2 mL	Alwsci Technologies (China)	-
SPE manifold	-	Supelco	SOP/C/151
Adapter for SPE	-	Baker	
Chromatograph	Prominence	Shimadzu Corp. (Japan)	SOP/C/304

Reagents and solvents

Chemical	Grade	Manufacturer/ Supplier	Batch Number	Expiry date
Deionized water	HPLC grade	Łukasiewicz-IPO*	Fresh prepared before analysis	
Ortho-phosphoric acid	85% HPLC	SUPELCO	Z0721828108	31.07.2023
Acetonitrile	HPLC	Chempur	220601232	06.2024
		VWR Chemicals	22E194018	19.05.2025
		Chempur	221005030	10.2024
Hydrochloric acid	ACS reagent	Sigma-Aldrich	STBK 0854	12.2023
			STBK7572	04.2024
Methanol	Pure p.a.	POCH	1344/02/21	02.2026
Acetone	Pure p.a.	POCH	1316/01/22	01.2026
sodium chloride	Pure p.a.	POCH	1253/12/19	12.2024
ethyl acetate	Pure p.a.	POCH	1238/05/21	05.2026
sodium sulfate anhydrous	99.4%	J.T.Baker	2106706810	08.03.2026
SPE column Supelclean ENVI-18	3 mL, 500 mg	Supelco	12878002	28.01.2027
			15383801	18.05.2027

* The main stages of water purification: pre-treatment (mechanical filter, activated carbon), deionisation (ion exchange resin). Water prepared with SolPure-7 water deionizer [SOP/C/91]

The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The analytical methods were developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection were determined.

The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1 [10] and Standard Operating Procedure SOP/C/9 . The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. During the validation process three methods was development. First method - dilution method with limit of quantification 0.5 mg/L for clopyralid, fluroxypyr-meptyl and 0.05 mg/L for florasulam. Second method – solid phase extraction (SPE) method with limit of quantification 0.001 mg/L for clopyralid and 0.0005 mg/L for

florasulam. Finally third method – liquid-liquid extraction (LLE) method with limit of quantification 0.001 mg/L for fluroxypyr-meptyl. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.
All fresh test item concentrations and the control, collected at exposure initiation and all spent test item concentrations and the control collected at the first renewal were chemically analysed. Moreover, fresh and spent samples of the highest test item concentration of 20.0 mg/L and the lowest test item concentration of 0.06 mg/L and the control during each renewal and at exposure termination were also chemically analysed.

Validation - Results and discussions

Table 5.2-32: Methods suitable for the determination of the residues in water for CHR/H/CFF 250 EC

	Residues
Author(s), year	E. Nierzędska, 2023
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Analyte	Working solution concentrations [mg/L]	range of linearity of calibration curve [mg/L]	equivalent calibration range of linearity [mg analyte/L]	
			dilution method	SPE/LLE method
clopyralid	0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0	0.05 – 5.0	0.1 – 10.0	0.0005 – 0.05
florasulam	0.01, 0.02, 0.05, 0.1, 0.2 and 0.5	0.01 – 0.5	0.02 – 1.0	0.0001 – 0.005
fluroxypyr-meptyl	0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0	0.05 – 5.0	0.1 – 10.0	0.0005 – 0.05
clopyralid	1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0	1.0 – 100.0	2.0 – 200.0	0.01 – 1.0
florasulam	0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0	0.1 – 10.0	0.2 – 20.0	0.001 – 0.1
fluroxypyr-meptyl	1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0	1.0 – 100.0	2.0 – 200.0	0.01 – 1.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL equivalent to mg/L (equal to µg/mL).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r²
0.05 – 5.0	clopyralid	123231	-830.816	0.9999220
0.01 – 0.5	florasulam	72211.4	-123.271	0.9997957
0.05 – 5.0	fluroxypyr-meptyl	110669	-903.182	0.9996382
1.0 – 100.0	clopyralid	122759	-132.833	0.9999876
0.1 – 10.0	florasulam	72890.5	-323.919	0.9999835
1.0 – 100.0	fluroxypyr-meptyl	110480	-646.597	0.9999819

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability

<table>
<tr><td></td><td>Residues</td></tr>
<tr><td></td><td>of the chosen function were demonstrated as the regression residual (di). The regression residual are presented in a residual plots in range equal to range of linearity of calibration curves.</td></tr>
<tr><td>Recovery and repeatability</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analyzed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the methods is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%. A summary of the recovery data of control and fortified samples are presented in thetable below
<table>
<tr><th>Analyte</th><th>Method</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">clopyralid</td><td rowspan="4">SPE method</td><td>0.001</td><td>5</td><td>101.0</td><td>6.9</td></tr>
<tr><td>0.01</td><td>5</td><td>87.3</td><td>9.9</td></tr>
<tr><td rowspan="2">florasulam</td><td>0.0005</td><td>5</td><td>90.0</td><td>8.9</td></tr>
<tr><td>0.005</td><td>5</td><td>99.8</td><td>4.8</td></tr>
<tr><td rowspan="2">fluroxypyr-meptyl</td><td rowspan="2">LLE method</td><td>0.001</td><td>5</td><td>109.0</td><td>2.8</td></tr>
<tr><td>0.01</td><td>5</td><td>98.8</td><td>3.6</td></tr>
<tr><td rowspan="2">clopyralid</td><td rowspan="5">Dilution method</td><td>0.5</td><td>5</td><td>105.0</td><td>1.3</td></tr>
<tr><td>5.0</td><td>5</td><td>102.4</td><td>0.2</td></tr>
<tr><td rowspan="2">florasulam</td><td>0.05</td><td>5</td><td>102.6</td><td>2.3</td></tr>
<tr><td>0.5</td><td>5</td><td>102.8</td><td>1.0</td></tr>
<tr><td rowspan="2">fluroxypyr-meptyl</td><td>0.5</td><td>5</td><td>78.2</td><td>6.1</td></tr>
<tr><td>5.0</td><td>5</td><td>80.0</td><td>0.5</td></tr>
</table></td></tr>
<tr><td>Selectivity</td><td>The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the methods were demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample</td></tr>
<tr><td>Matrix Effects (water)</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard preparing in solution to standard preparing in blank matrix at appropriate concentration. The matrix effect is not exceed ± 20 %. The matrix effect and concentration are presented in table below.
<table>
<tr><th>Analyte</th><th>Method</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>clopyralid</td><td rowspan="2">SPE method</td><td>0.1</td><td>-11.1</td></tr>
<tr><td>florasulam</td><td>0.05</td><td>15.1</td></tr>
<tr><td>fluroxypyr-meptyl</td><td>LLE method</td><td>0.1</td><td>17.0</td></tr>
<tr><td>clopyralid</td><td rowspan="3">dilution method</td><td>0.25</td><td>-0.5</td></tr>
<tr><td>florasulam</td><td>0.025</td><td>-2.0</td></tr>
<tr><td>fluroxypyr-meptyl</td><td>0.25</td><td>-2.7</td></tr>
</table></td></tr>
<tr><td>LOQ/LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LoD) is defined as the lowest detectable concentration or amount of an analyte in a sample.
Limit of quantification (LoQ) and a limit of detection (LoD) are presented in the table below.</td></tr>
</table>

	Residues

Analyte	Method	LoQ [mg analyte /L]	equivalent calibration level [µg/mL]	LoD [mg analyte/L]	equivalent calibration level [µg/mL]
clopyralid	SPE method	0.001	0.1	0.0005	0.05
florasulam		0.0005	0.05	0.0001	0.01
fluroxypyr-meptyl	LLE method	0.001	0.1	0.0005	0.05
clopyralid	dilution method	0.5	0.25	0.1	0.05
florasulam		0.05	0.025	0.02	0.01
fluroxypyr-meptyl		0.5	0.25	0.1	0.05

Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with LOQ an according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/08
Report	Validation of analytical method for determination of active substances florasulam, clopyralid and fluroxypyr (in the form of fluroxypyr-meptyl) of the test item CHR/H/CFF 250 EC in deionized water, M. Świstak, 2022, Study Code: 0038/0065/FA
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Validation of method was based on experimental procedure SPB-FA/11 and Guideline SANTE/2020/12830, rev.1. During the validation of the analytical method the following parameters: selectivity, matrix effects, linearity, accuracy, precision (repeatability), limit of detection and limit of quantification were determined. Determination of the active substances: florasulam, clopyralid and fluroxypyr (in the form of fluroxypyr-meptyl) of the test item in deionized water was performed by high performance liquid chromatography with PDA detection on the basis of signals from active substances. Identification of active substances was made by comparing the UV spectra and retention times of standards solution and solution of the test item.

Reagents and solutions
− florasulam standard, IPO Warszawa, lot number 2A/21 (certificate of analysis – Appendix 1)
− clopyralid standard, Sigma Aldrich, lot number BCCF3174 (certificate of analysis – Appendix 2)
− fluroxypyr-meptyl standard, IPO Warszawa, lot number 3B/21 (certificate of analysis – Appendix 3)

− acetonitrile, HPLC grade, Avantor, lot number 1277/5/2
− orthophosphoric acid p.a. 85%, Chempur, lot number 19/04/08
− deionized water
− ultrapure water
− 0.1% (v/v) orthophosphoric acid (prepared by adding 1.18 mL of orthophosphoric acid 85%, p.a. to 1000 mL volumetric flask filled with 900 mL of ultrapure water, and then filling up to the mark with ultrapure water)

Equipment
− high performance liquid chromatography Shimadzu Nexera series LC-30 with PDA detector
− analytical balance Radwag XA 82_220.4Y.A
− automatic pipettes: Transferpette S 10 µL, Transferpette S 200 µL, Transferpette S 1 mL, Transferpette S 5 mL
− deionizer SolPure 78
− system for obtaining ultrapure water Millipore Synergy UV
− ultrasonic washer Sonic-10
− volumetric flask class A
− syringes and syringe filters 0.22 µm

Validation - Results and discussions

Table 5.2-33: Methods suitable for the determination of the residues in wheat for fluroxypyr

	Residues
Author(s), year	M. Świstak, 2023
Principle of method	HPLC-UV
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Florasulam standard solution prepared according to point 4.4, clopyralid standard solution prepared according to point 4.5 and fluroxypyr-meptyl standard solution prepared according to point 4.6 were used to determine the linearity of the method. By dilution with acetonitrile, solutions of florasulam at concentration: 0.037 mg/L; 0.146 mg/L; 0.292 mg/L; 0.584 mg/L; 5.843 mg/L, solutions of clopyralid at concentration: 0.433 mg/L; 1.730 mg/L; 3.459 mg/L; 6.918 mg/L; 69.183 mg/L and solutions of fluroxypyr-meptyl at concentration: 0.626 mg/L; 2.503 mg/L; 5.006 mg/L; 10.011 mg/L; 100.110 mg/L were prepared. After analysis graph dependence of peak area from the active substances and fluroxypyr-meptyl to standard concentration was plotted and linear correlation coefficient was determined. Calibration curve is described by equation: for florasulam $f(x) = 28338.7 * x + 173.071$ for clopyralid $f(x) = 6273.65 * x - 305.306$ for fluroxypyr-meptyl $f(x) = 11688.1 * x - 56.0948$ Florasulam r = 0.999 (0.037 mg/L – 5.843 mg/L) Clopyralid r = 0.999 (0.433 mg/L – 69.183 mg/L) Fluroxypyr-meptyl

	Residues
	r = 0.999 (0.626 mg/L – 100.110 mg/L) A random distribution of regression residues was obtained
Selectivity	For selectivity analysis, the following analysis of samples were performed: mobile phase; acetonitrile; deionized water; florasulam, clopyralid and fluroxypyr-meptyl standards solution in acetonitrile (at the LOD level). The UV spectra of the active substance standard were recorded for comparison with the UV spectra of test item solution. Acceptance criteria were fulfilled: − no signals from other substances with area exceeding 30% of the LOQ area, in place of the active substance peak − the comparison of the UV spectra allows for the identification of active substances and fluroxypyr-meptyl.
Accuracy	To determine the accuracy of the method, determination of mixture of deionized water and acetonitrile in volume ratio 1:1 (in duplicate) and test item solutions prepared according to point 4.7 at two concentration levels each in five replicates were performed: − nominal concentrations for florasulam: level I = 0.182 mg/L, level II = 1.815 mg/L − nominal concentrations for clopyralid: level I = 2.166 mg/L, level II = 21.663 mg/L − nominal concentrations for fluroxypyr-meptyl: level I = 3.146 mg/L, level II = 31.458 mg/L. Accuracy of method equal: for florasulam: 105.22% − level I: 102.97%, level II: 107.46% for clopyralid: 95.90% − level I: 95.17%, level II: 96.62% for fluroxypyr-meptyl: 98.85% − level I: 98.23%, level II: 99.46%. Outlier was identified from the obtained results of the fluroxypyr-meptyl determination (level I – 2.998 mg/L) analysis using the Dixon test. The result was not included in the accuracy calculation. Criterion of acceptance was fulfilled: − mean recovery in the range of 70-120% at each fortification level − no more than one outlier occurs at each fortification level.
Precision	To determine precision of the method, the results of sample determinations prepared during the determination of accuracy, Precision of method equal: for florasulam − level I (average determined concentration 0.187 mg/L): 2.7% − level II (average determined concentration 1.950 mg/L): 0.6% for clopyralid − level I (average determined concentration 2.061 mg/L): 3.6% − level II (average determined concentration 20.932 mg/L): 0.4% for fluroxypyr-meptyl − level I (average determined concentration 3.090 mg/L): 0.4% − level II (average determined concentration 31.288 mg/L): 0.3%. Outlier was identified from the obtained results of the fluroxypyr-meptyl determination (level I – 2.998 mg/L) analysis using the Dixon test. The result was not included in the accuracy calculation. Criterion of acceptance: − RSD [%] ≤20% was fulfilled − no more than one outlier occurs at each fortification level.
Matrix Effects	Matrix effects equal: − florasulam: 0.4% − clopyralid: -2.8% − fluroxypyr meptyl: 5.7% The acceptance criterion: matrix effects do not exceed ±20% was fulfilled.
LOQ/LOD	The limit of detection (LOD) and the limit of quantification (LOQ) were determined when determining the linearity and accuracy of the method.

	Residues
	The limit of quantification is the nominal value of the concentration of the active substance in the test item solutions prepared according to point 4.7 at the lower level of the accuracy analysis. Acceptance criterion was fulfilled: LOD value below 30% of LOQ value. Limit of detection (LOD) equal: − 0.037 mg/L – for florasulam − 0.433 mg/L – for clopyralid − 0.626 mg/L – for fluroxypyr-meptyl. Limit of quantification (LOQ) equal: − 0.182 mg/L – for florasulam − 2.166 mg/L – for clopyralid − 3.146 mg/L – for fluroxypyr-meptyl.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/09
Report	CHR/H/CFF 250 EC Daphnia magna, Acute Immobilisation Test, Appendix 4. Validation of analytical method for clopyralid, florasulam and fluoxypyr-meptyl and chemical analysis, Z. Kaceprek-Karetta, 2023, Study Code: w-03-20
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Equipment

Equipment	Size, Description	Manufacturer/Supplier	Standard Operating Procedure
Analytical Balance	Adventurer Pro AV 114CM	Ohaus Corporation (USA)	SOP/C/122
Volumetric flasks	Various volumes	Glassco (Germany)	SOP/C/12
Variable volume single-channel pipettes	Various volumes	Eppendorf AG (Germany)	
Rotary vacuum evaporator with water medium bath	RV 05 basic HB 4 basic	IKA - WERKE (Germany)	SOP/C/118
Rotary vacuum evaporator with water medium bath	RV 10 digital HB 10 digital	IKA - WERKE (Germany)	SOP/C/329
Autosampler vials with PTFE/silicone septa and screw caps	Clear glass, 2 mL	Alwsci Technologies (China)	-
SPE manifold	-	Supelco	SOP/C/151
Adapter for SPE	-	Baker	
Chromatograph	Prominence	Shimadzu Corp. (Japan)	SOP/C/304

Reagents and solvents

Chemical	Grade	Manufacturer/Supplier	Batch Number	Expiry date
Deionized water	HPLC grade	Łukasiewicz-IPO*	Fresh prepared before analysis	
Ortho-phosphoric acid	85% HPLC	SUPELCO	Z0721828108	31.07.2023
Acetonitrile	HPLC	Chempur	220601232	06.2024
		VWR Chemicals	22E194018	19.05.2025
		Chempur	220803052	08.2024
Hydrochloric acid	ACS reagent	Sigma-Aldrich	STBK 0854	12.2023
			STBK7572	04.2024
Methanol	Pure p.a.	POCH	1344/02/21	02.2026
Acetone	Pure p.a.	POCH	1316/01/22	01.2026
SPE column Supelclean ENVI-18	3 mL, 500 mg	Supelco	12878002	28.01.2027
			15383801	18.05.2027

* The main stages of water purification: pre-treatment (mechanical filter, activated carbon), deionisation (ion exchange resin). Water prepared with SolPure-7 water deionizer [SOP/C/91]

The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The analytical methods were developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection were determined.

The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1 [1] and Standard Operating Procedure SOP/C/9. The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.

All fresh test item concentrations and the control, collected at exposure initiation and all spent test item concentrations collected at renewal were analysed. Moreover, fresh and spent samples of the highest test item concentration (with living daphnids), the lowest test item concentration, and the control at renewal and at exposure termination were analysed

Validation - Results and discussions

Table 5.2-34: Methods suitable for the determination of the residues in Elendt M7 for CHR/H/CFF 250 EC

<table>
<tr><td></td><td>Residues</td></tr>
<tr><td>Author(s), year</td><td>Z. Kacperek-Karetta, 2023</td></tr>
<tr><td>Principle of method</td><td>HPLC-DAD</td></tr>
<tr><td>Linearity (linear between mg/L) (correlation coefficient, expressed as r)</td><td>Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.
<table>
<tr><th>Analyte</th><th>Working solution concentrations [mg/L]</th><th>range of linearity of calibration curve [mg/L]</th><th>equivalent calibration range of linearity [mg/L Elendt M7]</th></tr>
<tr><td>clopyralid</td><td>0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0</td><td>0.05 – 5.0</td><td>0.0005 – 0.05</td></tr>
<tr><td>florsulam</td><td>0.01, 0.02, 0.05, 0.1, 0.2 and 0.5</td><td>0.01 – 0.5</td><td>0.0001 – 0.005</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0</td><td>0.05 – 5.0</td><td>0.0005 – 0.05</td></tr>
<tr><td>clopyralid</td><td>1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0</td><td>1.0 – 100.0</td><td>0.01 – 1.0</td></tr>
<tr><td>florsulam</td><td>0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0</td><td>0.1 – 10.0</td><td>0.001 – 0.1</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0</td><td>1.0 – 100.0</td><td>0.01 – 1.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linear was given in µg/mL equivalent to mg/L (equal to µg/mL).
<table>
<tr><th>range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.05 – 5.0</td><td>clopyralid</td><td>123231</td><td>-830.816</td><td>0.9999220</td></tr>
<tr><td>0.01 – 0.5</td><td>florsulam</td><td>72211.4</td><td>-123.271</td><td>0.9997957</td></tr>
<tr><td>0.05 – 5.0</td><td>fluroxypyr-mepthyl</td><td>110669</td><td>-903.182</td><td>0.9996382</td></tr>
<tr><td>1.0 – 100.0</td><td>clopyralid</td><td>122759</td><td>-132.833</td><td>0.9999876</td></tr>
<tr><td>0.1 – 10.0</td><td>florsulam</td><td>72890.5</td><td>-323.919</td><td>0.9999835</td></tr>
<tr><td>1.0 – 100.0</td><td>fluroxypyr-mepthyl</td><td>110480</td><td>-646.597</td><td>0.9999819</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function were demonstrated as the regression residual (di). The regression residual are presented in a residual plots in range equal to range of linearity of calibration curves.</td></tr>
<tr><td>Recovery and repeatability</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analyzed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the methods is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.</td></tr>
</table>

Residues

Analyte	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]
clopyralid	0.001	5	108.0	2.8
	0.01	5	82.7	2.5
florsulam	0.0005	5	96.0	4.2
	0.005	5	97.0	0.8
fluroxypyr-mepthyl	0.001	5	106.0	15.1
	0.01	5	83.2	12.0

Selectivity

The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the methods were demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample

Matrix Effects (Elendt M7)

Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard preparing in solution to standard preparing in blank matrix at appropriate concentration

Analyte	Concentration [mg/L]	Matrix effect [%]
clopyralid	0.1	5.5
florasulam	0.05	-8.8
fluroxypyr-mepthyl	0.1	-4.8

LOQ/LOD

Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample.

Analyte	LOQ [mg analyte /L]	equivalent calibration level [µg/mL]	LOD [mg analyte/L]	equivalent calibration level [µg/mL]
clopyralid	0.001	0.1	0.0005	0.05
florsulam	0.0005	0.05	0.0001	0.01
fluroxypyr-mepthyl	0.001	0.1	0.0005	0.05

Comment

The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

zRMS: Method is accepted as method for generation pre-authorization data.

Reference:	KCP 5.1.1/10
Report	CHR/H/CFF 250 EC Earthworm reproduction test (Eisenia andrei), Appendix No. 2. Results of analytical measurements, P. Pieczka, 2023, Study Code: G-01-20
Guideline(s):	SANTE/2020/12830, Rev.2
Deviations:	NO
GLP:	YES
Acceptability:	YES

Materials and methods:

Equipment

Equipment	Size, Description	Manufacturer/Supplier	Standard Operating Procedure
Analytical Balance	Adventurer Pro AV 114CM	Ohaus Corporation (USA)	SOP/C/122
Balance	WPS 510/C	Radwag (Poland)	SOP/C/25
Volumetric flasks	Various volumes	Glassco (Germany)	SOP/C/12
Variable volume single-channel pipettes	Various volumes	Eppendorf AG (Germany)	
Ultrasonic bath	Sonic-5	Polsonic	SOP/C/332
Laboratory timer	-	TFA Dostmann GmbH & Co. KG	-
Laboratory centrifuge	MPW-351e	MPW Med. Instruments	SOP/C/361
Autosampler vials with PTFE/silicone septa and screw caps	Clear glass, 2 mL	Alwsci Technologies (China)	-
Chromatograph	Prominence	Shimadzu Corp. (Japan)	SOP/C/304

Reagents and solvents

Chemical	Grade	Manufacturer/Supplier	Batch Number	Expiry date
Deionized water	HPLC grade	Łukasiewicz-IPO*	Fresh prepared before analysis	
Ortho-phosphoric acid	85% HPLC	SUPELCO	Z0721828108	31.07.2023
Acetonitrile	HPLC	Chempur	220601232	06.2024
		Chempur	221005030	10.2024
		Chempur	221012016	10.2024
		Chempur	221019007	10.2024

* The main stages of water purification: pre-treatment (mechanical filter, activated carbon), deionisation (ion exchange resin). Water prepared with SolPure-7 water deionizer [SOP/C/91]

The concentration of active substances of test item was chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The analytical methods were

developed for the determination of active substances of test item in matrix. The range of linearity of the analytical graph, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The validated analytical methods were performed according to SANTE/2020/12830, Rev. 1 [1] and Standard Operating Procedure SOP/C/9. The concentrations of clopyralid, florasulam and fluroxypyr-meptyl were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/304, SOP/C/531]. The concentrations of fluroxypyr-meptyl were stoichiometrically recalculated and expressed as fluroxypyr (acid) concentrations.

Validation - Results and discussions

Table 5.2-35: Methods suitable for the determination of the residues in artificial soil for CHR/H/CFF 250 EC

	Residues
Author(s), year	P. Pieczka, 2023
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Analyte	Working solution concentrations [mg/L]	range of linearity of calibration curve [mg/L]	equivalent calibration range of linearity [mg/kg artificial soil]
clopyralid	0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0	0.05 – 5.0	0.05 – 5.0
florsulam	0.01, 0.02, 0.05, 0.1, 0.2 and 0.5	0.01 – 0.5	0.01 – 0.5
fluroxypyr-mepthyl	0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0	0.05 – 5.0	0.05 – 5.0
clopyralid	1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0	1.0 – 100.0	1.0 – 100.0
florsulam	0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0	0.1 – 10.0	0.1 – 10.0
fluroxypyr-mepthyl	1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0	1.0 – 100.0	1.0 – 100.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linear was given in µg/mL equivalent to mg/L (equal to µg/mL).

range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.05 – 5.0	clopyralid	123231	-830.816	0.9999220
0.01 – 0.5	florsulam	72211.4	-123.271	0.9997957
0.05 – 5.0	fluroxypyr-mepthyl	110669	-903.182	0.9996382
1.0 – 100.0	clopyralid	122759	-132.833	0.9999876
0.1 – 10.0	florsulam	72890.5	-323.919	0.9999835
1.0 – 100.0	fluroxypyr-mepthyl	110480	-646.597	0.9999819

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the

<table>
<tr><th></th><th>Residues</th></tr>
<tr><td></td><td>chosen function were demonstrated as the regression residual (di). The regression residual are presented in a residual plots in range equal to range of linearity of calibration curves.</td></tr>
<tr><td>Recovery and repeatability</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analyzed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the methods is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Analyte</th><th>Fortification Level [mg/kg]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">clopyralid</td><td>1.0</td><td>5</td><td>89.2</td><td>1.8</td></tr>
<tr><td>10.0</td><td>5</td><td>75.8</td><td>0.5</td></tr>
<tr><td rowspan="2">florsulam</td><td>0.1</td><td>5</td><td>98.6</td><td>13.5</td></tr>
<tr><td>1.0</td><td>5</td><td>90.3</td><td>0.6</td></tr>
<tr><td rowspan="2">fluroxypyr-mepthyl</td><td>1.0</td><td>5</td><td>73.7</td><td>2.8</td></tr>
<tr><td>10.0</td><td>5</td><td>89.6</td><td>0.8</td></tr>
</table></td></tr>
<tr><td>Selectivity</td><td>The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the methods were demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample</td></tr>
<tr><td>Matrix Effects (Artificial soil)</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard preparing in solution to standard preparing in blank matrix at appropriate concentration
<table>
<tr><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>clopyralid</td><td>1.0</td><td>16.7</td></tr>
<tr><td>florasulam</td><td>0.1</td><td>2.9</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>1.0</td><td>-14.2</td></tr>
</table></td></tr>
<tr><td>LOQ/LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample.
<table>
<tr><th>Analyte</th><th>LOQ [mg analyte /kg]</th><th>equivalent calibration level [µg/mL]</th><th>LOD [mg analyte/kg]</th><th>equivalent calibration level [µg/mL]</th></tr>
<tr><td>clopyralid</td><td>1.0</td><td>1.0</td><td>0.05</td><td>0.05</td></tr>
<tr><td>florsulam</td><td>0.1</td><td>0.1</td><td>0.01</td><td>0.01</td></tr>
<tr><td>fluroxypyr-mepthyl</td><td>1.0</td><td>1.0</td><td>0.05</td><td>0.05</td></tr>
</table></td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830, Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of all analytes in all matrices with an LOQ according to the guidance document(s) SANTE/2020/12830, Rev.2.. With regard to selectivity, accuracy

and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study,

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted