REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.06001.H.2.B

Product name(s): EDAPTIS

Chemical active substances:

Mesosulfuron-methyl, 12 g/L
Pinoxaden, 60 g/L

Safener:

Mefenpyr-diethyl, 35 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Sponsor: ADAMA Agan Ltd.
Applicant: Country organisation / representative of ADAMA, as given in Part A

Submission date: June 2021, updated September 2022, April 2023

MS Finalisation date: April 2023 (initial Core Assessment)

August 2023, December 2023 (final Core Assessment)

Version history

When	What
June 2021	First version submitted by applicant
September 2022	Clarification regarding analytical methods for pinoxaden added
April 2023	Analytical methods for pinoxaden in water added
April 2023	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
August 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant is ~~struck through~~ and shaded.
December 2023	Final report (Core Assessment updated following the second commenting period) No additional information or assessments after the second commenting period.

DATA PROTECTION CLAIM

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A

STATEMENT FOR OWNERSHIP

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5 Analytical methods

This application under article 33 of regulation 1107/2009 submitted by the applicant in June 2021 is for first authorisation of the product ADM.06001.H.2.B (containing 12 g/L mesosulfuron-methyl, 60 g/L pinoxaden and 35 g/L mefenpyr-diethyl (safener)) follows the data requirements of

- Regulation (EC) No. 544/2011 for the active substance pinoxaden,
- Regulation (EC) No. 283/2013 for the active substance mesosulfuron-methyl, and
- Regulation (EC) No. 284/2013 for the plant protection product ADM.06001.H.2.B.

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

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gap is:

- None
- ~~analytical method for monitoring residues of pinoxaden in body fluids with the LOQ of 0.01 mg/L.~~

Commodity/crop	Supported/Not supported
Winter wheat, rye, triticale	supported
Spring wheat	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 mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl (safener) in plant protection product Mesosulfuron-methyl 12 g/L + Pinoxaden 60 g/L + Mefenpyr-diethyl 35 g/L OD (ADM.06001.H.2.B) is provided as follows:

Comments of zRMS:	The analytical method was successfully validated for the determination of Mesosulfuron-methyl, Pinoxaden and Mefenpyr-diethyl in plant protection product according to the requirements laid down by SANCO3030/99 rev.5.

Reference:	KCP 5.1.1/01 also filed under KCP 2.1/01
Report	Determination of Storage Stability and Physical-Chemical Properties of Mesosulfuron-methyl 12 g/l + Pinoxaden 60 g/l + Mefenpyr-diethyl 35 g/l OD (ADM.06001.H.2.B) Stored at 54°C for 14 Days and at 0°C for 7 Days Tsesin N., 2020 Report no.: 000105084, 000105084.069FL Including 1st and 2nd Amendment to report, 2021
Guideline(s):	EU SANCO/3030/99 rev. 5, 22/03/19 considering Brazilian Standard ABNT NBR 14029 3d Ed. 09/12/2016 Australian Pesticides & Veterinary Medicines Authority Guidelines, (EC) SANCO No. 1107/2009 - Final draft OPPTS: 830.1800 and Series 830, Group B 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The validation of the analytical method permits the determination of the active ingredients (mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl) in Mesosulfuron-methyl 12 g/L + Pinoxaden 60 g/L + Mefenpyr-diethyl 35 g/L OD (ADM.06001.H.2.B) formulation. The quantification was performed by HPLC-DAD using external standard technique.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuron-methyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L
Matrix Blank	-	BLA8001	-
Mesosulfuron-methyl	208465-21-8	232-3283	99.4%
Pinoxaden	243973-20-8	257-3521	99.3%
Mefenpyr-diethyl	135590-91-9	114-2079	99.5%

Sample preparation

About 300 mg (for AIs determination) of the formulation were weighed into a 50 mL volumetric flask. About 30 ml acetone were added as a solvent and solutions were mixed well. After the solutions reached the room temperature acetone was added up to the mark and the solutions were mixed well and measured by injection of a 10 µl aliquots of these solutions into the HPLC/DAD. The calibration standard solutions were injected in the same sequence.

HPLC-DAD Conditions for pinoxaden, mesosulfuron-methyl and mefenpyr-diethyl determination

HPLC-DAD system	Hewlett-Packard 1100 Series equipped with an autosampler, column oven and degasser, Diode array detector, Xcalibrur data system
Column	Synergi 4 µm Hydro-RP 80 Å, 250x4.6mm ID
Column Temperature	40 °C
Injection Volume	10 µL
Flow rate	1.0 mL/min
Eluent	Eluent A: acetonitrile Eluent B: 0.1% phosphoric acid in water
Gradient	

Time (minutes)	Eluent A (%)	Eluent B (%)
0	25	75
5	25	75
8	40	60
12	50	50
22	55	45
30	100	0
34	100	0

Detector	DAD, 244 nm for mesosulfuron-methyl and pinoxaden analysis and 290 nm for mefenpyr-diethyl analysis
Retention time (s)	Mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl elutes at 15, 25 and 28 minutes respectively.

Validation - Results and discussions

Repeatability

Two repeatability assays were performed by two operators. A.i. Repeatability (assay 1; 69FL-14-1- 69FL to 14-5) study results (first operator), a.i.s Repeatability (assay 2; 69FL-16-1 to 69FL-16-5) study results (second operator).

Table 5.2-1: Method precision - method repeatability 1 and 2

Sample ID no.	Batch A8001	Mesosulfuron-methyl		Pinoxaden		Mefenpyr-diethyl	
	Weight mg	Area	RF[1]	Area	RF[1]	Area	RF[1]
69FL-14-1	311.2	1734.45569	5.573	4589.29248	14.747	3255.73389	10.462
69FL-14-2	331.5	1853.54443	5.591	4929.25488	14.870	3489.27417	10.526
69FL-14-3	308.6	1717.28760	5.565	4551.89111	14.750	3235.60742	10.485
69FL-14-4	307.0	1724.72937	5.618	4572.51025	14.894	3255.74072	10.605
69FL-14-5	299.8	1622.51001	5.412	4330.18115	14.444	3099.25073	10.338
RF = Area / Weight		Mean SD RSD	5.552 0.081 1.456		14.741 0.179 1.216		10.483 0.098 0.933
69FL-16-1	302.7	1705.54700	5.634	4516.78027	14.922	3327.12378	10.991
69FL-16-2	296.0	1645.82483	5.560	4373.28369	14.775	3171.44238	10.714
69FL-16-3	298.3	1672.04846	5.605	4443.31201	14.895	3200.91479	10.731
69FL-16-4	297.8	1669.11182	5.605	4423.96143	14.855	3199.81494	10.745
69FL-16-5	306.4	1711.57263	5.586	4543.52100	14.829	3264.82593	10.655
RF = Area / Weight		Mean SD RSD	5.598 0.027 0.489		14.855 0.058 0.387		10.767 0.130 1.206

[1] RF - Response Factor

The obtained repeatability RSD values are less than the threshold values of ≤2.61% for mesosulfuron-methyl, ≤2.03% for pinoxaden and ≤2.19% for mefenpyr-diethyl, acceptable for ~1.2%, ~6.3% and ~3.8% analyte concentrations for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl, respectively,

according to SANCO guidelines. Therefore, it can be concluded that the analytical method has a good analytical method repeatability (precision).

The relative standard deviation of the RF obtained for a.i´s from 10 injections from two assays was taken as the indication of analytical method intermediate precision.

Table 5.2-2: Intermediate precision study results (assay 1 and assay 2)

Sample ID no.	Batch A8001	Mesosulfuron-methyl		Pinoxaden		Mefenpyr-diethyl	
	Weight mg	Area	RF[1]	Area	RF[1]	Area	RF[1]
69FL-14-1	311.2	1734.45569	5.573	4589.29248	14.747	3255.73389	10.462
69FL-14-2	331.5	1853.54443	5.591	4929.25488	14.870	3489.27417	10.526
69FL-14-3	308.6	1717.28760	5.565	4551.89111	14.750	3235.60742	10.485
69FL-14-4	307.0	1724.72937	5.618	4572.51025	14.894	3255.74072	10.605
69FL-14-5	299.8	1622.51001	5.412	4330.18115	14.444	3099.25073	10.338
69FL-16-1	302.7	1705.54700	5.634	4516.78027	14.922	3327.12378	10.991
69FL-16-2	296.0	1645.82483	5.560	4373.28369	14.775	3171.44238	10.714
69FL-16-3	298.3	1672.04846	5.605	4443.31201	14.895	3200.91479	10.731
69FL-16-4	297.8	1669.11182	5.605	4423.96143	14.855	3199.81494	10.745
69FL-16-5	306.4	1711.57263	5.586	4543.52100	14.829	3264.82593	10.655
RF = Area / Weight		Mean SD RSD	5.575 0.062 1.110		14.798 0.139 0.941		10.625 0.185 1.740

[1] RF - Response Factor

The obtained repeatability RSD values are less than the threshold values, calculated by Horwitz equation, ≤3.89% for mesosulfuron- methyl, ≤3.03% for pinoxaden and ≤3.27% for mefenpyr-diethyl acceptable for ~1.2%, ~6.3% and ~3.8% analyte concentrations for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl respectively according to Brazilian standard ABNT NBR 14029, 3rd. Therefore, it can be concluded that the analytical method has a good analytical method intermediate precision.

Accuracy

To three sets of two matrix blank samples containing appropriate amount of materials, mesosulfuron-methyl, pinoxaden, and mefenpyr-diethyl standards were added at maximal, medium and minimal concentration levels in final solutions.

Table 5.2-3: Accuracy analysis results at maximal concentration level

Sample ID no.	Weight, mg of matrix blank	Area in matrix blank	Cu [mg/mL]	Nominal C_{Sample} [mg/mL]	C u [g/kg]			
69FL-21-BL1	146.9	0.00000	0.00000	5.876	0.00			
69FL-21-BL2	158.2	0.00000	0.00000	6.328	0.00			
		Area in sample	C_F [mg/mL]	Nominal C_{Sample} [mg/mL]	C_A [g/kg]	C_F [g/kg]	Recovery [%]	Mean Recovery RSD [%]
Mesosulfuron-methyl		**At Max concentration level spiked (C_A): 0.1225 mg/mL**						
69FL-21-1	131.9	2805.81348	0.12271	5.276	23.2109	23.2577	100.20	100 0.45
69FL-21-1	131.9	2785.83228	0.12183	5.276	23.2109	23.0920	99.46	
69FL-21-2	133.3	2798.32910	0.12238	5.332	22.9671	22.9520	99.93	
69FL-21-2	133.3	2777.74780	0.12148	5.332	22.9671	22.7832	99.20	
Pinoxaden		**At Max concentration level spiked (C_A): 0.4760 mg/mL**						
69FL-21-1	131.9	5792.79297	0.48513	5.276	90.2207	91.9494	101.92	101 0.68
69FL-21-1	131.9	5747.64941	0.48134	5.276	90.2207	91.2328	101.12	
69FL-21-2	133.3	5707.29932	0.47797	5.332	89.2732	89.2328	100.41	
69FL-21-2	133.3	5785.39893	0.48451	5.322	89.2732	90.8676	101.79	
Mefenpyr-diethyl		**At Max concentration level spiked (C_A): 0.2658 mg/mL**						
69FL-21-1	131.9	3877.07446	0.26690	5.276	50.3761	50.5875	100.42	100 0.51
69FL-21-1	131.9	3840.60571	0.26439	5.276	50.3761	50.1116	99.47	
69FL-21-2	133.3	3886.15088	0.26752	5.332	49.8470	50.1733	100.65	
69FL-21-2	133.3	3863.91943	0.26599	5.332	49.8470	49.8863	100.08	

Table 5.2-4: Accuracy analysis results at nominal concentration level

Sample ID	Weight, mg of matrix blank	Area in matrix blank	Cu [mg/mL]	Nominal C Sample [mg/mL]	C u [g/kg]			
69FL-21-BL1	146.9	0.00000	0.00000	5.876	0.00			
69FL-21-BL2	158.2	0.00000	0.00000	6.328	0.00			
		Area in sample	**C F [mg/mL]**	**Nominal C Sample [mg/mL]**	**C_A [g/kg]**	**C_F [g/kg]**	**Recovery [%]**	**Mean Recovery RSD [%]**
Mesosulfuron-methyl		**At Max concentration level spiked (CA): 0.0735 mg/mL**						
69FL-21-3	138.7	1674.53552	0.07323	5.548	13.2438	13.1999	99.67	100
69FL-21-3	138.7	1684.95520	0.07369	5.548	13.2438	13.2820	100.29	0.52
69FL-21-4	134.1	1694.62085	0.07411	5.364	13.6981	13.8164	100.86	
69FL-21-4	134.1	1691.24670	0.07396	5.364	13.6981	13.7889	100.66	
Pinoxaden		**At Max concentration level spiked (C_A): 0.3570 mg/mL**						
69FL-21-3	138.7	4276.65527	0.35815	5.548	64.3481	64.5555	100.32	101
69FL-21-3	138.7	4299.26611	0.36005	5.548	64.3481	64.8968	100.85	0.78
69FL-21-4	134.1	4351.73682	0.36444	5.364	66.5554	67.9422	102.08	
69FL-21-4	134.1	1332.87939	0.36286	5.364	66.5554	67.6478	101.64	
Mefenpyr-diethyl		**At Max concentration level spiked (C_A): 0.2161 mg/mL**						
69FL-21-3	138.7	3191.21338	0.21968	5.548	38.9535	39.5971	101.65	102
69FL-21-3	138.7	3167.87183	0.21808	5.548	38.9535	39.3074	100.91	0.99
69FL-21-4	134.1	3178.50391	0.21881	5.364	40.2897	40.7922	101.25	
69FL-21-4	134.1	3239.62256	0.22302	5.364	40.2897	41.5766	103.19	

Table 5.2-5: Accuracy analysis results at minimal concentration level

Sample ID	Weight, mg of matrix blank	Area in matrix blank	Cu [mg/mL]	Nominal C Sample [mg/mL]	C u [g/kg]			
69FL-21-BL1	146.9	0.00000	0.00000	5.876	0.00			
69FL-21-BL2	158.2	0.00000	0.00000	6.328	0.00			
		Area in sample	**C F [mg/mL]**	**Nominal C Sample [mg/mL]**	**C_A [g/kg]**	**C_F [g/kg]**	**Recovery [%]**	**Mean Recovery RSD [%]**
Mesosulfuron-methyl		**At Max concentration level spiked (C_A): 0.0480 mg/mL**						
69FL-21-5	138.0	1113.27795	0.04869	5.520	8.7011	8.8202	101.37	101
69FL-21-5	138.0	1103.41248	0.04826	5.520	8.7011	8.7420	100.47	0.65
69FL-21-6	136.0	1116.71753	0.04884	5.440	8.8291	8.9775	101.68	
69FL-21-6	136.0	1120.22485	0.04899	5.440	8.8291	9.0057	102.00	
Pinoxaden		**At Max concentration level spiked (C_A): 0.2456 mg/mL**						
69FL-21-5	138.0	2972.93506	0.24897	5.520	44.4979	45.1037	101.36	102
69FL-21-5	138.0	3054.13062	0.25577	5.520	44.4979	46.3355	104.13	1.33
69FL-21-6	136.0	3013.45947	0.25237	5.440	45.1523	46.3908	102.74	
69FL-21-6	136.0	2968.78906	0.24863	5.440	45.1523	45.7032	101.22	
Mefenpyr-diethyl		**At Max concentration level spiked (C_A): 0.1519 mg/mL**						
69FL-21-5	138.0	2247.29614	0.15470	5.520	27.5139	28.0262	101.86	102
69FL-21-5	138.0	2237.60986	0.15404	5.520	27.5139	27.9054	101.42	0.25
69FL-21-6	136.0	2245.81689	0.15460	5.440	27.9185	28.4197	101.80	
69FL-21-6	136.0	2250.62036	0.15493	5.440	27.9185	28.4805	102.01	

C_F – Concentration of fortified sample (g/kg), $C_F = C_F$ (mg/ml) ×1000/C_{sample} (mg/ml)
C_U – Concentration of unfortified sample (g/kg), $C_U = C_U$ (mg/ml) ×1000/C_{sample} (mg/ml)
C_A – Concentration of added sample (g/kg), $C_A = C_A$ (mg/ml) ×1000/C_{sample} (mg/ml)
C_F (mg/ml) – Experimental concentration of AI in sample with standard addition, CF (mg/ml) = Area (AI peak area) / A (Calibration equation coefficient)
C_U (mg/ml) – Experimental concentration of AI in blank sample, C_U (mg/ml) = Area / A
C_A (mg/ml) – Theoretical concentration of AI in mg/ml, which was added into the solution.
C_{sample} (mg/ml) – Experimental concentration of matrix blank in sample

Table 5.2-6: Summary of accuracy analysis at different concentration levels

Active substance	Active substance concentration level, %	Mean recovery (%)		Precision, (RSD %)	
		SANCO Acceptance criteria	Found	Acceptance criteria	Found
Mesosulfuron-methyl	Maximal, 2.0	90 – 110	100	2.41	0.45
	Medium, 1.2		100	2.61	0.52
	Minimal, 0.8	80 – 120	101	2.77	0.65

Pinoxaden	Maximal, 8.4	90 - 110	101	1.95	0.68
	Medium, 6.3		101	2.03	0.78
	Minimal, 4.4		102	2.14	1.33
Mefenpyr-diethyl	Maximal, 4.6		100	2.13	0.51
	Medium, 3.8		102	2.19	0.99
	Minimal, 2.7		102	2.31	.025

Table 5.2-7: Methods suitable for the determination of active substances mesosulfuron-methyl, pinoxaden and safener mefenpyr-diethyl in plant protection product Mesosulfuron-methyl 12 g/l + Pinoxaden 60 g/l +Mefenpyr-diethyl 35 g/l OD (ADM.06001.H.2.B)

	Mesosulfuron-methyl	**Pinoxaden**	**Mefenpyr-diethyl**
Author(s), year	Tsesin N., 2020		
Principle of method	HPLC-DAD	HPLC-DAD	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Six levels in the range of ~ 0.02 – 0.20 mg/mL R = 1.0000	Six levels in the range of ~ 0.10 – 0.70 mg/mL R = 0.9999	Six levels in the range of ~ 0.10 – 0.35 mg/mL R = 0.9998
Precision – Repeatability Mean, n = 5, (%RSD)	0.59%	0.50 %	0.52%
System – Repeatability Mean, n = 5, (%RSD)	0.39%	0.34 %	0.37%
Accuracy Two samples at three levels injected in duplicate (% Recovery)	Mean recovery (REC), % and RSD, %		

Maximum		**Medium**		**Minimum**	
REC	**RSD**	**REC**	**RSD**	**REC**	**RSD**
Pinoxaden					
99	0.38	99	0.34	99	0.44
Mesosulfuron-methyl					
99	0.24	99	0.06	99	0.53
Mefenpyr-diethyl					
100	0.34	100	0.15	100	0.45

Interference/ Specificity	Interference were <3% of total peak area for target analyte		
Comment	Acceptable	Acceptable	Acceptable

Conclusion

The analytical method for the determination of mesosulfuron-methyl, pinoxaden and safener mefenpyr-diethyl in plant protection product Mesosulfuron-methyl 12 g/L + Pinoxaden 60 g/L +Mefenpyr-diethyl 35 g/L OD (ADM.06001.H.2.B) was fully validated with regard to linearity, precision, accuracy and specificity. Validation acceptance criteria are based on guidance for generation and reporting methods of analysis in support of data requirements of SANCO/3030/99 rev. 5. The method is acceptable.

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

According to Final Renewal report for **mesosulfuron** = mesosulfuron-methyl, SANTE/11827/2016 Rev 2, 23 March 2017: No relevant impurities.

According to review report for **pinoxaden**, SANCO/11794/2013 rev 3, 29 January 2016 the impurity toluene is of toxicological concern and must not exceed the max. content of 1 g/kg.

Safener **mefenpyr-diethyl**: According to LoEP of Mefenpyr-diethyl (October 2011): Identity of relevant impurities: open

Therefore, an analytical method for determination of toluene in the product ADM.06001.H.2.B is provided with this application and summarised in the following. 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 proposed analytical method has been properly described and validated for the determination of toluene in the plant protection product in accordance with the requirements of SANCO/3030/99 rev.5.

Reference:	KCP 5.1.1/02
Report	Validation of the analytical method for determination of toluene in MESOSULFURON-METHYL 12 g/L + PINOXADEN 60 g/L + MEFENPYR-DIETHYL 35 g/L OD (ADM.06001.H.2.B) Ricau H., 2020 Report No. 20-901066-037, ADAMA reference no. 000106124
Guideline(s):	Yes, SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The objective of this study was to develop and validate an analytical method for the determination of the impurity toluene in the product ADM.06001.H.2.B. Toluene was analysed after extraction from the formulation and quantified by gas chromatography using a flame ionisation detector.

Pinoxaden is the relevant active substance of the formulation and its nominal value is 60 g/L.
As impurity, toluene nominal concentration should not exceed 1 g/kg pinoxaden in ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuron-methyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Toluene	108-88-3	K50002425	99.9%

Preparation of the test item solution
A quantity of 1.0 g of the test item was weighed (to the nearest 0.01 mg) into a 20-mL volumetric flask and the volume was made up with acetone. The solution was manually stirred before analysis.
For precision, 5 independent solutions were prepared (20-084 2B to 20-084 2F).

Preparation of the formulation blank solution
A quantity of 0.89 g of the formulation blank (20-084-3) was weighed (to the nearest 0.01 mg) into a 20-

mL volumetric flask and the volume was made up with acetone. The solution was manually stirred before analysis (20-084 BF A).

Preparation of the solutions for the accuracy study

At 0.003%

A quantity of 1 g of the test item was weighed into a 20-mL volumetric flask. A volume of 0.5 mL of the RCA1561-4 solution was added and made up with acetone (20-084 REC A).

At 0.006%

A quantity of 1 g of the test item was weighed into a 20-mL volumetric flask, A volume of 1.0 mL of the RCA1561-4 solution was added and filled made up with acetone (20-084 REC B).

At 0.012%

A quantity of 1 g of the test item was weighed into a 20-mL volumetric flask. A volume of 2.0 mL of the RCA1561-4 solution was added and made up with acetone (20-084 REC C).

At LOQ level (0.0006% w/w)

A quantity of 0.89 g of the formulation blank was weighed into a 20-mL volumetric flask. A volume of 0.4 mL of the RCA1561-2 solution was added and made up with acetone. Two solutions were prepared (20-084 LOQ A and 20-084 LOQ B).

Preparation of RCA1561-4: 12.0 mg reference item dissolved in 200 mL acetone.
Preparation of RCA1561-2: 3.6 mg reference item dissolved in 250 mL acetone.

GC-FID chromatographic conditions for toluene determination

GC	S.P.I
Injector	Volume injected: 1µL Temperature: 260°C
Column	Supelco, SPB-1, 30 m x 0.53 mm, 1.5µm
Oven program	Initial temperature: 50°C, Hold time: 10 min Rate: 50 °C/min to 300°C Hold time: 4 min
Detector	FID Temperature: 280 °C
Gas	Carrier gas: Helium, Flow rate: 6 mL/min Make-up: Nitrogen, Flow rate 25mL/min Detector gas: Air, Flow rate 300 mL/min Hydrogen: Flow rate: 30 mL/min
Run Time:	19 min
Retention time	About 7 min

Validation - Results and discussions

Table 5.2-8: Methods suitable for the determination of the relevant impurity toluene in ADM.06001.H.2.B

	Toluene
Author(s), year	Ricau, H., 2020
Principle of method	Extraction with acetone and analysis via GC-FID
Linearity	Linear response within the range of 0.33 mg/L to 10.57 mg/L (6 levels) y = 1.16E+02 x C – 1.13E+01, r = 0.9997
Precision – Repeatability Mean n =5 (%RSD)	The concentration of toluene in the test item was equal to 0.000699% w/w. In this case, the precision was acceptable as the R.S.D. was lower than the result of the modified Horwitz equation: 3.35 < 8.00 (C = 0.00000699) with Horwitz ratio (Horrat) equal to 0.42.
Accuracy n = 5 (% Recovery)	For formulations containing less than 0.01% of an impurity, the recovery results should be in the range 70% - 130% and they were experimentally equal to 116.8% at 0.003%; 108.1% at 0.006% and 103.2% at 0.012%.

	Toluene
	The recovery result was experimentally equal to 111.4% at LOQ level.
Interference/ Specificity	No interference was observed in the solvent blank, the blank formulation, the reference item and the test item at the retention time of toluene (<30 % of LOQ)
LOQ	Limit of quantification (LOQ) of toluene was determined to be 0.00057% w/w in the test item.
LOD	Limit of detection (LOD) of toluene was estimated at 0.00017% w/w in the test item.
Comment	Acceptable

Conclusion
The analytical method fulfils the requirements of SANCO/3030/99 rev.5 and is suitable for the determination of toluene in the plant protection product ADM.06001.H.2.B.

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

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

CIPAC No 663.201 (**mesosulfuron-methyl**) and CIPAC No 663 (mesosulfuron) available according to Final Renewal report for mesosulfuron (variant evaluated mesosulfuron-methyl) SANTE/11827/2016 Rev 2, 23 March 2017

Pinoxaden: CIPAC method 776 is available but not published.

Mefenpyr-diethyl: CIPAC No. 651.229, Content Handbook N. The method is usable for TC, WG, OD, EW, EC-formulations.

5.2.2 Methods for the determination of residues of mesosulfuron-methyl (KCP 5.1.2)

The plant protection product Mesosulfuron-methyl 12 g/L + Pinoxaden 60 g/L +Mefenpyr-diethyl 35 g/L OD (ADM.06001.H.2.B) contains the active substances mesosulfuron-methyl and pinoxaden, and the safener mefenpyr-diethyl.

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

Table 5.2-9: Validated methods for the generation of pre-authorization data for mesosulfuron-methyl

Component of residue definition for plant matrices (EFSA, 2016): Mesosulfuron-methyl **Component of residue definition for animal matrices (EFSA, 2016):** Not required for intended uses				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content *Wheat whole plant* High starch content *Wheat grain* ~~No group~~ Dry commodities *Wheat straw* (Residues)	Primary method B19G-A4-M-03 Mesosulfuron-methyl	0.01 mg/kg	LC-MS/MS	KCP 5.1.2/01 Barbier G., 2019 Report: B19G-A4-M-03 ADAMA reference: 000102681 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Animal products, food of animal origin (Residues)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water, sediment (Environmental fate)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water (Efficacy)	No additional data.			
	Confirmatory (if required)	--	--	--
Feed, body fluids (Toxicology)	No additional data.			
	Confirmatory (if required)	--	--	--
Body fluids, air (Exposure)	No additional data.			
	Confirmatory (if required)	--	--	--
Water, buffer solutions (Properties)	No additional data.			
	Confirmatory (if required)	--	--	--

Table 5.2-10: Validated methods for the generation of pre-authorization data for mesosulfuron-methyl (Ecotoxicology)

Component of residue definition (EFSA, 2016):
Soil: Mesosulfuron-methyl, mesosulfuron (AE F154851), AE F160459, AE F092944, AE F160460, AE F1405844, AE F1447447
Surface water: - Mesosulfuron-methyl, mesosulfuron (AE F154851), AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447, BCS-CV14885, BCS-CO60720
Groundwater: Mesosulfuron-methyl, mesosulfuron (AE F154851), AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447, BCS-CV14885
Air: Mesosulfuron-methyl

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Elendt M4 medium *Daphnia magna* acute toxicity (OECD 202) (Ecotoxicology)	Primary method Mesosulfuron-methyl	0.2 mg test item/L	LC-MS/MS	KCP 5.1.2/05 filed under KCP 10.2.1/01 Seidel U. and Mollandin G., 2021a Report no: 140711220, ADAMA no: 000105363 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
OECD 201 test medium *Raphidocelis subcapitata (=Pseudo-kirchneriella subcapitata)* - algal growth inhibition test) (OECD 201) (Ecotoxicology)	Primary Mesosulfuron-methyl	0.05 mg test item/L	LC-MS/MS	KCP 5.1.2/06 filed under KCP 10.2.1/02 Seidel U. and Mollandin G., 2021b Report no: 140711210 ADAMA no: 000105364 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
20x AAP growth medium *Lemna gibba* - toxicity to higher aquatic plants (OECD 221) (Ecotoxicology)	Primary Mesosulfuron-methyl	0.005 mg test item/L	LC-MS/MS	KCP 5.1.2/07 filed under KCP 10.2.1/03 Seidel U. and Mollandin G., 2021c Report no: 140711240 ADAMA no: 000105365 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- 50 % (w/v) aqueous sucrose solution (diet) Honey Bee - Chronic Toxicity Test (OECD 245) (Ecotoxicology)	Primary Mesosulfuron-methyl	1.8 g test item/L	LC-MS/MS	KCP 5.1.2/08 filed under KCP 10.3.1.2/01 Sekine T. and Kowalczyk F., 2021 Report no: 140711136, ADAMA no: 000105367 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Honey Bee - Larvae chronic toxicity test (OECD 239) (Ecotoxicology)	Primary Mesosulfuron-methyl	6.37 mg/L	LC-MS/MS	KCP 5.1.2/09 filed under KCP 10.3.1.3/01 Colli M., 2020, Report no: BT138/20 Version 2 ADAMA no: 000105368 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

Component of residue definition (EFSA, 2016):
Soil: Mesosulfuron-methyl, mesosulfuron (AE F154851), AE F160459, AE F092944, AE F160460, AE F1405844, AE F1447447
Surface water: - Mesosulfuron-methyl, mesosulfuron (AE F154851), AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447, BCS-CV14885, BCS-CO60720
Groundwater: Mesosulfuron-methyl, mesosulfuron (AE F154851), AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447, BCS-CV14885
Air: Mesosulfuron-methyl

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Earthworms - sub-lethal effects (OECD 222) (Ecotoxicology)	Primary Mesosulfuron-methyl	10 mg/kg dry soil	LC-MS/MS	KCP 5.1.2/10 filed under KCP 10.4.1.1/01 Straube D. and Gourlay V., 2021, Report no: 140711022 ADAMA no: 000105375 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Seedling Emergence and Seedling Growth Test (OECD 208) (Ecotoxicology)	Primary Mesosulfuron-methyl	1.0 g test item /L	LC-MS/MS	KCP 5.1.2/11 filed under KCP 10.6.2/01 Spatz B. and Kowalczk, 2021a, Report no: 140711086 ADAMA no: 000105379 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Vegetative vig-our test (OECD 227) (Ecotoxicology)	Primary Mesosulfuron-methyl	1.0 g test item /L	LC-MS/MS	KCP 5.1.2/12 filed under KCP 10.6.2/02 Spatz B. and Kowalczk, 2021b, Report no: 140711087 ADAMA no: 000105380 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

5.2.3 Methods for the determination of residues of pinoxaden (KCP 5.1.2)

Pinoxaden / Pre-registration methods

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

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

Component of residue definition for plant matrices (EFSA, 2013):
Sum of M4 and M6 expressed as parent pinoxaden (to include free and conjugated residues of M4 and M6)
Component of residue definition for animal matrices (EFSA, 2013 and EFSA 2021):
(1) None necessary as a result of the representative use; however M4 would be the most suitable component for ruminant matrices based on exposure resulting from the representative use in cereals **(EFSA, 2013)**
(2) M4 (free and conjugated), expressed as pinoxaden **(EFSA, 2021**)
Component of residue definition (EFSA, 2013):
Soil: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Surface water: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56
Groundwater: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Air: Pinoxaden

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) *Wheat grain* High water content *Wheat whole plant* ~~No group~~ Dry commodities *Wheat straw* (Residues)	Primary Method REM 199.02 Metabolites NOA407854 (M2), SYN 505164 (M4), SYN 502836 (M6), SYN 505887 (M10)	0.01 mg/kg for grain 0.02 mg/kg for straw and whole plant	LC-MS/MS Extraction by reflux with HCl (Hydrolysis)	Gasser A., 2002a Report no: REM 199.02 Gasser A., 2002b Report no: 02-S302 *EU agreed (DAR Volume 3, Annex B, B.1 – B.5, July 2006)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
High protein/high starch content (dry) *Barley grain* High water content *Barley whole plant* ~~No group~~ Dry commodities *Barley straw* (Residues)	Primary Method REM 199.03 Metabolites NOA407854 (M2), SYN 505164 (M4), SYN 502836 (M6), SYN 505887 (M 10)	0.01 mg/kg for grain 0.02 mg/kg for straw and whole plant	LC-MS/MS Off line SPE, extraction by reflux with HCl	Crook S.J., 2004 Report no: REM 199.03 *EU agreed (DAR Volume 3, Annex B, B.1 – B.5, July 2006)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
High protein/high starch content (dry) *Wheat grain* High water content *Wheat whole plant* ~~No group~~ Dry commodities *Wheat straw* (Residues)	Primary method B19G-A4-P-05 Pinoxaden metabolites M4 and M6	0.01 mg/kg for M4 wheat grain M6 wheat grain and straw 0.02 mg/kg for M4 in whole plant and straw and M6 in whole plant.	LC-MS/MS	KCP 5.1.2/02 Barbier G., 2020 Report: B19G-A4-P-05 ADAMA reference: 000102680 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High protein/high starch content (dry) *Wheat grain*	Modification of method B19G-A4-P-05	0.01 mg/kg for M4 in wheat grain M6 in wheat grain and straw	LC-MS/MS	KCP 5.1.2/03 Meric D., 2021 Report: DMC-20-42727 ADAMA reference: 000105437

Component of residue definition for plant matrices (EFSA, 2013):
Sum of M4 and M6 expressed as parent pinoxaden (to include free and conjugated residues of M4 and M6)
Component of residue definition for animal matrices (EFSA, 2013 and EFSA 2021):
(1) None necessary as a result of the representative use; however M4 would be the most suitable component for ruminant matrices based on exposure resulting from the representative use in cereals **(EFSA, 2013)**
(2) M4 (free and conjugated), expressed as pinoxaden **(EFSA, 2021**)
Component of residue definition (EFSA, 2013):
Soil: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Surface water: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56
Groundwater: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Air: Pinoxaden

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
~~No group~~ Dry commodities *Wheat straw* (Residues)	Pinoxaden metabolites M4 and M6	0.02 mg/kg for M4 in straw.		**See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS or LC-MS^3)	
Animal products, food of animal origin (Residues)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water, sediment (Environmental fate)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water (Efficacy)	No additional data.			
	Confirmatory (if required)	--	--	--
Feed, body fluids (Toxicology)	No additional data.			
	Confirmatory (if required)	--	--	--
Body fluids, air (Exposure)	No additional data.			
	Confirmatory (if required)	--	--	--
Water, buffer solutions (Properties)	No additional data.			
	Confirmatory (if required)	--	--	--

Table 5.2-12: Validated methods for the generation of pre-authorization data for pinoxaden (Ecotoxicology)

Component of residue definition (EFSA, 2013):
Soil: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Surface water: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56
Groundwater: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Air: Pinoxaden

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Elendt M4 medium *Daphnia magna* acute toxicity (OECD 202) (Ecotoxicology)	Primary Pinoxaden and Pinoxaden M2	0.2 mg test item/L for pinoxaden and. 2 µg pinoxaden M2/L	LC-MS/MS	KCP 5.1.2/05 filed under KCP 10.2.1/01 Seidel U. and Mollandin G., 2021a Report no: 140711220, ADAMA no: 000105363

Component of residue definition (EFSA, 2013):
Soil: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Surface water: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56
Groundwater: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Air: Pinoxaden

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				See Appendix 2
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
OECD 201 test medium *Raphidocelis subcapitata (=Pseudo-kirchneriella subcapitata)* - algal growth inhibition test) (OECD 201) (Ecotoxicology)	Primary Pinoxaden and Pinoxaden M2	0.05 mg test item/L for pinoxaden and 1.5 µg pinoxaden M2/L	LC-MS/MS	KCP 5.1.2/06 filed under KCP 10.2.1/02 Seidel U. and Mollandin G., 2021b Report no: 140711210 ADAMA no: 000105364 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
20x AAP growth medium *Lemna gibba* - toxicity to higher aquatic plants (OECD 221) (Ecotoxicology)	Primary Pinoxaden and Pinoxaden M2	0.005 mg test item/L for pinoxaden and 0.72 µg pinoxaden M2/L.	LC-MS/MS	KCP 5.1.2/07 filed under KCP 10.2.1/03 Seidel U. and Mollandin G., 2021c Report no: 140711240 ADAMA no: 000105365 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- 50 % (w/v) aqueous sucrose solution (diet) Honey Bee - Chronic Toxicity Test (OECD 245) (Ecotoxicology)	Primary Pinoxaden	1.8 g test item/L	LC-MS/MS	KCP 5.1.2/08 filed under KCP 10.3.1.2/01 Sekine T. and Kowalczyk F., 2021 Report no: 140711136, ADAMA no: 000105367 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Honey Bee - Larvae chronic toxicity test (OECD 239) (Ecotoxicology)	Primary Pinoxaden and 51.49 µg/L Pinoxaden M2	33.45 mg/L Pinoxaden and 51.49 µg/L Pinoxaden M2	LC-MS/MS	KCP 5.1.2/09 filed under KCP 10.3.1.3/01 Colli M., 2020, Report no: BT138/20 Version 2 ADAMA no: 000105368 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Seedling Emergence and Seedling Growth Test (OECD 208) (Ecotoxicology)	Primary Pinoxaden	1.0 g test item /L	LC-MS/MS	KCP 5.1.2/11 filed under KCP 10.6.2/01 Spatz B. and Kowalczk, 2021a, Report no: 140711086 ADAMA no: 000105379 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

Component of residue definition (EFSA, 2013):
Soil: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Surface water: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56
Groundwater: Pinoxaden, NOA 407854 (M2), NOA 447204 (M3)
Air: Pinoxaden

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
- deionized water Vegetative vigour test (OECD 227) (Ecotoxicology)	Primary Pinoxaden	1.0 g test item /L	LC-MS/MS	KCP 5.1.2/12 filed under KCP 10.6.2/02 Spatz B. and Kowalczk, 2021b, Report no: 140711087 ADAMA no: 000105380 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

5.2.4 Methods for the determination of residues of mefenpyr-diethyl (KCP 5.1.2) Mefenpyr-diethyl / Pre-registration methods

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

Table 5.2-13: Validated methods for the generation of pre-authorization data for mefenpyr-diethyl - methyl

Component of residue definition for plant matrices (Austria 2011):
Cereal grain: Mefenpyr-diethyl (AE F107892) and metabolite AE F094270 expressed as mefenpyr-diethyl.
Cereal shoot and straw: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F109453 and AE F094270 expressed as mefenpyr-diethyl
Component of residue definition for animal matrices (Austria/France: LoEP, October 2011):
Mefenpyr diethyl (AE F107892) and metabolite AE F113225 expressed as mefenpyr diethyl.
Component of residue definition (Austria/France: LoEP, October 2011):
Soil: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE 2211046
Surface water: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE F109453, AE F114952, AE 2211046
Groundwater: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE 2211046
Air: Mefenpyr-diethyl (AE F107892)

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High protein/high starch content (dry) *Wheat grain* High water content *Wheat whole plant* ~~No group~~ Dry commodities *Wheat straw* (Residues)	Primary method B19S-A4-M-01 Mefenpyr-diethyl and metabolite, 1-(2,4-dichloro-phenyl)-5-methyl pyrazole-3-carboxylic acid	0.01 mg/kg	LC-MS/MS QuEChERS	KCP 5.1.2/04 Lefresne S., 2019 Report: B19S-A4-M-01 ADAMA reference: 000102679 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Animal products, food of animal origin, ... (Residues)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water, sediment (Environmental fate)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water (Efficacy)	No additional data.			
	Confirmatory (if required)	--	--	--
Feed, body fluids (Toxicology)	No additional data.			
	Confirmatory (if required)	--	--	--
Body fluids, air (Exposure)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water (Ecotoxicology)	No additional data.			
	Confirmatory (if required)	--	--	--
Water, buffer	No additional data.			

Component of residue definition for plant matrices (Austria 2011): **Cereal grain**: Mefenpyr-diethyl (AE F107892) and metabolite AE F094270 expressed as mefenpyr-diethyl. **Cereal shoot and straw**: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F109453 and AE F094270 expressed as mefenpyr-diethyl **Component of residue definition for animal matrices (Austria/France: LoEP, October 2011):** Mefenpyr diethyl (AE F107892) and metabolite AE F113225 expressed as mefenpyr diethyl. **Component of residue definition (Austria/France: LoEP, October 2011):** **Soil:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE 2211046 **Surface water:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE F109453, AE F114952, AE 2211046 **Groundwater:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE 2211046 **Air:** Mefenpyr-diethyl (AE F107892)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
solutions (Properties)	Confirmatory (if required)	--	--	--

Table 5.2-14: Validated methods for the generation of pre-authorization data for mefenpyr-diethyl - methyl (Ecotoxicology)

Component of residue definition (Austria/France: LoEP, October 2011): **Soil:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE 2211046 **Surface water:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE F109453, AE F114952, AE 2211046 **Groundwater:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE 2211046				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Elendt M4 medium *Daphnia magna* acute toxicity (OECD 202) (Ecotoxicology)	Primary Mefenpyr-diethyl	0.2 mg test item/L	LC-MS/MS	KCP 5.1.2/05 filed under KCP 10.2.1/01 Seidel U. and Mollandin G., 2021a Report no: 140711220, ADAMA no: 000105363 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
OECD 201 test medium *Raphidocelis subcapitata (=Pseudo-kirchneriella subcapitata)* - algal growth inhibition test) (OECD 201) (Ecotoxicology)	Primary Mefenpyr-diethyl	0.05 mg test item/L	LC-MS/MS	KCP 5.1.2/06 filed under KCP 10.2.1/02 Seidel U. and Mollandin G., 2021b Report no: 140711210 ADAMA no: 000105364 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
20x AAP growth medium *Lemna gibba* - toxicity to higher aquatic plants (OECD 221) (Ecotoxicology)	Primary Mefenpyr-diethyl	0.005 mg test item/L	LC-MS/MS	KCP 5.1.2/07 filed under KCP 10.2.1/03 Seidel U. and Mollandin G., 2021c Report no: 140711240 ADAMA no: 000105365 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

Component of residue definition (Austria/France: LoEP, October 2011): **Soil:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE 2211046 **Surface water:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE F109453, AE F114952, AE 2211046 **Groundwater:** Mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F094270, AE 2211046				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
- 50 % (w/v) aqueous sucrose solution (diet) Honey Bee - Chronic Toxicity Test (OECD 245) **(Ecotoxicology)**	Primary Mefenpyr-diethyl	3.6 g test item/L	LC-MS/MS	KCP 5.1.2/08 filed under KCP 10.3.1.2/01 Sekine T. and Kowalczyk F., 2021 Report no: 140711136, ADAMA no: 000105367 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Honey Bee - Larvae chronic toxicity test (OECD 239) **(Ecotoxicology)**	Primary Mefenpyr-diethyl	20.18 mg/L	LC-MS/MS	KCP 5.1.2/09 filed under KCP 10.3.1.3/01 Colli M., 2020 Report no: BT138/20 Version 2 ADAMA no: 000105368 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Seedling Emergence and Seedling Growth Test (OECD 208) (Ecotoxicology)	Primary Mefenpyr-diethyl	1.0 g test item /L	LC-MS/MS	KCP 5.1.2/11 filed under KCP 10.6.2/01 Spatz B. and Kowalczk, 2021a, Report no: 140711086 ADAMA no: 000105379 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
- deionized water Vegetative vig-our test (OECD 227) (Ecotoxicology)	Primary Mefenpyr-diethyl	1.0 g test item /L	LC-MS/MS	KCP 5.1.2/12 filed under KCP 10.6.2/02 Spatz B. and Kowalczk, 2021b, Report no: 140711087 ADAMA no: 000105380 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

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

Mesosulfuron-methyl
With regard to the monitoring enforcement methods (post-registration methods) for mesosulfuron-methyl reference is made to the unprotected analytical methods available at EU level. For summary, a reference is made to the data compiled in the RAR 2016 and Original Annex II submission, the Final renewal report of mesosulfuron SANTE/11827/2016 Rev 2 and the EFSA Journal 2016;14(10):4584. Besides, additional studies are referenced where this is relevant.

Pinoxaden
With regard to the monitoring enforcement methods (post-registration methods) for pinoxaden reference is made to the unprotected analytical methods available at EU level. For summary, a reference is made to the data compiled in the Final renewal report of pinoxaden, SANCO/11794/2013 rev 3, (January 2016) and the EFSA Journal 2013;11(8):3269. Besides, additional studies are referenced where this is relevant.

Mefenpyr-diethyl (safener)
With regard to the monitoring enforcement methods (post-registration methods) for mefenpyr-diethyl reference is made to the unprotected analytical methods available at EU level.
For summary, a reference is made to the data compiled in the Draft Assessment Report mefenpyr-diethyl (2011), the Addenda 1 to the DAR (finalised September) and the Addenda 2 to the DAR (finalised December 2013). Besides, additional studies are referenced where this is relevant.

These data are considered to provide the relevant dossier information on the active substances.

5.3.1 Analysis of the plant protection product (KCP 5.2)

Please refer to the analytical methods for the determination of the active substance and relevant impurities in the plant protection product as provided in chapter 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of mesosulfuron-methyl (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Mesosulfuron methyl	0.01 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Plant, high acid content		0.01 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Plant, high oil content		~~0.02~~ 0.01 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Plant, difficult matrices (hops, spices, tea)		0.02 mg/kg for herbs and edible flowers 0.05 mg/kg for teas, coffee, herbal infusions, cocoa and carobs	Lowest MRL MRL Regulation (EU) No 289/2014
Muscle	Mesosulfuron-methyl	0.02 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Milk		0.02 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Eggs		0.02 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Fat		0.02 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Liver, kidney		0.02 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Soil (Ecotoxicology)	Mesosulfuron-methyl	0.05 mg//kg	Common limit
Drinking water (Human toxicology)	Mesosulfuron-methyl	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Mesosulfuron-methyl	*Lemna gibba:* NOEC: 0.00039 mg/L	EFSA Conclusion 4584/2016

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Air	Mesosulfuron-methyl	12 µg/m^3	AOEL sys/AOEL inhal: 0.13 mg/kg bw/d
Tissue (meat or liver)	Mesosulfuron-methyl	0.02 mg/kg	Lowest MRL MRL Regulation (EU) No 289/2014
Body fluids		0.01 mg/L	Common limit

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

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: Mesosulfuron-methyl (Regulation (EU) No 289/2014)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content *Wheat shoots* High starch content *Wheat grain* ~~No group~~ Dry commodities *Wheat straw*	Primary method DGM F02/99-0 AE F130060	0.01 mg/kg in grain (wheat) 0.05 mg/kg in straw and shoots (wheat)	LC-MS/MS	Method and validation Wrede A., 1999 Doc. No. C005129 *EU agreed (Original Annex II submission)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High water content *Cereal shoots* High starch content *Cereal grain* ~~No group~~ Dry commodities *Cereal straw*	Primary method EM F08/99-0 AE F130060	0.01 mg/kg in grain 0.05 mg/kg in straw and shoots	LC-MS/MS	Method Wrede A., 2000i Doc. No.C006734 *EU agreed (Original Annex II submission)*
		0.01 mg/kg in grain 0.05 mg/kg in straw and shoots	LC-MS/MS	Validation Wrede A., 2000 Doc. No. C006735 *EU agreed (Original Annex II submission)*
		0.01 mg/kg in grain 0.05 mg/kg in straw and shoots	LC-MS/MS	Validation Wrede A.; 2000 Doc. No. C008827 *EU agreed (Original Annex II submission)*
	ILV	0.01 mg/kg in grain	LC-MS/MS	Reichert N.;2000c; Doc. No.C009586 *EU agreed (Original Annex II submission)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High starch content *Cereal grain*	Primary method EM F02/00-0	0.05 mg/kg in grain	LC-MS/MS	Wrede A., 2000 Doc. No. C009496

Component of residue definition: Mesosulfuron-methyl (Regulation (EU) No 289/2014)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	AE F130060			*EU agreed (Original Annex II submission)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High starch content *Cereal grain*	Primary method Multiresidue method	--	Statement	Wrede A., 2000 Doc. No. C009649 *EU agreed (Original Annex II submission)*
High water content *Tomato* High acid content *Lemon* High starch content *Maize kernel*	Primary method EM F 08/99-0 AE F130060	0.01 mg/kg	LC-MS/MS	Wrede A., 2002 Report no. C022220 Doc No. M-212674-01-1 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
High water content *Cereal shoots* ~~No group~~ Dry commodities *Cereal straw*	ILV Method EM F 08/99-0 AE F130060	0.05 mg/kg	LC-MS/MS	Reichert N., 2001 Report no. C011938 Doc No. M-201813-01 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
High water content *Tomato* High acid content *Citrus*	ILV Method EM F 08/99-0 AE F130060	0.01 mg/kg	LC-MS/MS	Reichert, N.; Klimmek, S.;2002 Report no: C023679 Doc no: M-215456-01 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High water content *Wheat shoots* *Flax w/o root* High starch content *Wheat grain* ~~No group~~ Dry commodities *Wheat straw* High oil content *Flax seed, oil, pomace*	Primary method 00815/M001 AE F130060	0.05 mg/kg Wheat (shoot, straw) Flax (plant without root) 0.01 mg/kg Wheat grain Flax (grain, oil, pomace wet)	LC-MS/MS	Heinemann O, 2004 Report no: 00815/M001 Doc no: M-226888-01 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High water content *Sugar beet root, sugar beet leaf* High acid content *Lemon* High oil content *Oilseed rape* ~~No group~~ Dry commodities *Cereal straw*	Primary method 01360	0.01 mg/kg	LC-MS/MS QuEChERS method	Stuke S., Ballmann C., 2013 Report no: MR-13/007 Doc no: M-455564-01 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

Component of residue definition: Mesosulfuron-methyl (Regulation (EU) No 289/2014)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content *Sugar beet root, sugar beet leaf* High acid content *Lemon* High oil content *Oilseed rape* ~~No group~~ Dry commodities *Cereal straw*	ILV	0.01 mg/kg	LC-MS/MS QuEChERS method	Konrad, S.;2013 Report no: 2013/0060/01 Doc No. M-470160-01 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High starch content *Wheat grain*	Primary 01360/M001	0.01 mg/kg	LC-MS/MS QuEChERS method	Stuke S., 2015 Report no: MR-15/090 Doc No: M-537921-01-1 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

No new data are presented in Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Wrede A., 2001, Report no. 02F021, Doc No: M-201883-01, EU agreed *(RAR 2016, Volume 3 – B.5 (AS))* Three accountability samples of shoot and straw, two shoot and straw control sample and three recoveries of samples of shoot and straw were extracted in parallel. The total mean radioactive residue (sum of radioactive residue in filter cake and extractable fraction) was 0.0052 mg equiv./kg in shoot and 0.0097 mg equiv./kg in straw. In shoot approximately 88% of the TRR (total radioactive residue) were extracted, 12% remained non-extractable (filter cake). In straw approximately 66% of the TRR (total radioactive residue) were extracted, 34% remained non-extractable (filter cake). Because of the small residues no radio HPLC and no determination by LC/MS/MS of the extracts was possible. The metabolism study gave an extractability of 87.2% TRR in shoot and 67.4 % TRR in straw. Therefore, the extraction efficiency of the residue analytical method EM F08/99–0 used as a monitoring method in plant is considered to be sufficiently demonstrated. Stuke S., 2015, Report no: MR-15/036/007, Doc no: M-525863-01-1 *EU agreed (RAR 2016, Volume 3 – B.5 (AS)), Cross validation* The method 01360 based on the official "QuEChERS-method" shows the same extraction efficiency as the extraction procedure applied during metabolism studies with ^{14}C-radioactive labelled mesosulfuron-methyl. Extraction efficiency is demonstrated for high water content crops. For dry commodities, since residues are very low (<0.01mg/kg), no more data are required.
Not required, because:	--

zRMS comments:
According to the EFSA Journal 2016;14(10):4584 *Monitoring mesosulfuron-methyl residues in food and feed of plant origin can be done by liquid chromatography with tandem mass spectrometry (LC–MS/MS) with limit of quantifications (LOQs) of 0.01 mg/kg in all commodity groups.*
No additional data are required.

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

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in animal matrices is given in the following tables. No new or additional studies were submitted.

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

Component of residue definition: Not required for intended uses – if required a default residue definition could be set as mesosulfuron-methyl only (EFSA, 2016)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Animal tissues (meat, fat, liver, kidney), egg, and milk	Primary method 01208/ M001	0.01 mg/kg	HPLC-MS/MS	Schmeer, K., Philipowski, C.; 2010 amended in 2011 Report no: 01208/M001, Doc no: M-389788-03-1 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	ILV method 01208/ M001	0.01 mg/kg	HPLC-MS/MS	Netzband D., 2010 Report no: RAMML014-01 Doc no: M-398300-02-1 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

No new data are presented in Appendix 2.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Based on animal metabolism data, residues of mesosulfuron-methyl are not anticipated to be above the LOQ in any matrix.

zRMS comments:
According to the EFSA Journal 2016;14(10):4584 *Adequate LC–MS/MS analytical method for monitoring residues of mesosulfuron-methyl in food and feed of animal origin is available with LOQs of 0.01 mg/kg in muscle, fat, milk, egg, liver and kidney.*
No additional data are required.

5.3.2.4 Description of methods for the analysis of mesosulfuron-methyl in soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron methyl in soil is given in the following tables. No new or additional studies were submitted.

Table 5.3-6: Validated methods for mesosulfuron-methyl (AE F130060) in soil

Component of residue definition: mesosulfuron-methyl (AE F130060)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Soil	Primary method DGM F05/970	2 µg/kg	HPLC/UV	Wrede A., 2000t Doc No: C009151

Component of residue definition: mesosulfuron-methyl (AE F130060)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	AE F130060			*EU agreed (Original Annex II submission)*
Soil	Data generation method DGM F04/99-0 AE F130060	1 µg/kg	LC-MS/MS	Wrede A., 2000n Doc No: C008681 *EU agreed (Original Annex II submission)*
	Validation DGM F04/99-0 AE F130060	1 µg/kg	LC-MS/MS	Wrede A., 2000m Doc No: C008682 *Original Annex II submission*
Soil	Enforcement method EM F13/99-0 AE F130060	0.01 µg/kg	LC-MS/MS	Wrede A., 2000b Doc No: C006394 *EU agreed (Original Annex II submission)*
	Validation EM F13/99-0 AE F130060	0.01 µg/kg	LC-MS/MS	Wrede A., 2000e Doc No: C009563 *EU agreed (Original Annex II submission)*
Soil (Höfchen and Laacher Hof)	Primary and confirmatory method 01115 AE F130060	0.1 µg/kg	HPLC-MS/MS	Freitag T. 2008, amended: 2013 Report no: M310074-03-1 *EU agreed (RAR 2016 Volume 3 – B.5 (AS))*

No new data are presented in Appendix 2.

zRMS comments:
According to the EFSA Journal 2016;14(10):4584 *LC–MS/MS methods are available enabling the determination of mesosulfuron-methyl residues in soil with LOQ of 0.1 µg/kg.*
No additional data are required.

5.3.2.5 Description of methods for the analysis of mesosulfuron-methyl in water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in surface and drinking water is given in the following tables. No new or additional studies were submitted.

Table 5.3-7: Validated methods for mesosulfuron-methyl in water

Component of residue definition: mesosulfuron-methyl (AE F130060)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water Ground water Surface water	Primary and confirmatory AE F130060	0.1 µg/L drinking water 0.1 µg/L ground water 0.5 µg/L surface water	HPLC-UV	Wrede A., 2000 Report No. C008689 *EU agreed (Original Annex II submission)*
Drinking water Ground water	Primary and confirmatory	0.2 µg/L drinking water	HPLC-UV	Wrede A., 2000 Report No. C008686

Component of residue definition: mesosulfuron-methyl (AE F130060)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface water	AE F130060	0.1 µg/L ground water 0.5 µg/L surface water		*EU agreed (Original Annex II submission)*
Drinking and Surface water	Enforcement method EM F04/00-0 AE F130060	0.05 µg/L	LC-MS/MS	Method Wrede A.,2001 Report No: C011206 Validation Wrede A., Neuss B., 2001 Report No: C011207 *EU agreed (RAR 2016, Volume 3 – B.5 (AS))*
Surface water	Analytical method 01387 AE F130060	0.05 µg/L	LC-MS/MS	Krebber R., Braune M., 2013 Report no: MR-13/085 *EU agreed (RAR 2016, Volume 3 – B.5 (AS))*
Surface water	ILV - Analytical methods 01333 and 01387 AE F130060	0.05 µg/L	LC-MS/MS	Stanislowski T., 2013 Report no: P3117 G *EU agreed (RAR 2016, Volume 3 – B.5 (AS))*

No new data are presented in Appendix 2.

zRMS comments:
According to the EFSA Journal 2016;14(10):4584 *LC–MS/MS methods are available enabling the determination of mesosulfuron-methyl residues in water with LOQ of 0.05 µg/L.*
The ILV for surface water with LOQ of 0.05 µg/L is available and acceptable.
No additional data are required.

5.3.2.6 Description of methods for the analysis of mesosulfuron-methyl in air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron methyl in air is given in the following tables. mesosulfuron-methyl. No new or additional studies were submitted.

Table 5.3-8: Validated methods for mesosulfuron methyl in air

Component of residue definition: mesosulfuron-methyl (AE F130060)				
Matrix	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Air	Primary AE F130060	12 µg/m^3	HPLC-UV	Reichert N., 2000 Report No.: C009587 *EU agreed (Original Annex II submission)*

No new data are presented in Appendix 2.

zRMS comments:
According to the EFSA Journal 2016;14(10):4584 *Residues of mesosulfuron-methyl in air can be determined by*

high-pressure/performance liquid chromatography-ultraviolet (HPLC-UV) with a LOQ of 12 µg/m^3.
No additional data are required.

5.3.2.7 Description of methods for the analysis of mesosulfuron-methyl in body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mesosulfuron-methyl in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: Mesosulfuron-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Animal tissues (meat, liver, kidney)	Primary method 01208/ M001	0.01 mg/kg	HPLC-MS/MS	Schmeer, K., Philipowski, C.; 2010 amended in 2011 Report no: 01208/M001 Doc no: M-389788-03-1 *EU agreed (RAR 2016, Volume 3 – B.5 (AS))*
Human urine	Primary and confirmatory	0.01 mg/kg L	QuEChERS (LC-MS/MS)	KCP 5.2/01 Watson G., 2021 ADAMA no: 000106703 **New data**

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

zRMS comments:
According to the SANTE/2020/12830, Rev.1, 24. February 2021 analytical methods for monitoring residues in body fluids and tissues with the LOQ of 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues should be validated. The Applicant submitted an analytical method for the determination of mesosulfuron-methyl in body fluids. The method is sufficient for the determination of mesosulfuron-methyl in human urine with LOQ of 0.01 mg/L.
More details please refer to Appendix 2.

5.3.2.8 Other studies/ information

No other studies and information are submitted in the framework of this application.

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

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	(1) ~~Pinoxaden (Reg. (EC) No 839/2008)~~ Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346) (2) Sum of M4 and M6 expressed as parent pinoxaden (to include free and conjugated residues of M4 and M6); provisionally (EFSA, 2013) M6 (free metabolite) only has been proposed as enforcement residue definition for plant products (cereals). However, the peer review did not come to a final agreement (EFSA, 2013) (3) Cereal crop group (option 1): sum of M4 and M6 (both free and conjugated), expressed as pinoxaden Cereal crop group (option 2): sum of M4 and M6 (both free only), expressed as pinoxaden (EFSA, 2021)	~~0.02~~ 0.03*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Plant, high acid content		~~0.02~~ 0.03*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Plant, high protein/high starch content (dry commodities)		~~0.05~~ 0.03*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Plant, high oil content		~~0.02~~ 0.03*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Plant, difficult matrices (hops, spices, tea)		~~0.05~~ 0.1*mg/kg for hops, teas, coffee, herbal infusions, cocoa and carobs	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Muscle	1) ~~Pinoxaden (Reg. (EC) No 839/2008)~~ Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346) (2) None necessary as a result of the representative use; however M4 would be the most suitable component for ruminant matrices based on exposure resulting from the representative use in cereals (EFSA, 2013) (3) M4 (free and conjugated), expressed as pinoxaden (EFSA, 2021)	~~No MRL~~ 0.02*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Milk		~~No MRL~~ 0.01*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Eggs		~~No MRL~~ 0.02*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Fat		~~No MRL~~ 0.02*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Liver, kidney		~~No MRL~~ 0.02*mg/kg	Lowest MRL ~~REG. (EC) No 839/2008~~ Reg. (EU) 2022/1346
Soil (Ecotoxicology)	Pinoxaden, NOA 407854 (M2) and NOA 447204 (M3)	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Pinoxaden, NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56)	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Pinoxaden NOA 407854 (M2)	*Crassostrea virginica* EC50 = > 0.88 mg a.s./L mm *Daphnia magna* NOEC = 6.25 mg/L nom	EFSA Conclusion 3269/2013
Air	Pinoxaden	1 µg/m^3	AOEL sys/AOEL inhal: 0.1 mg/kg bw/d

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Tissue (meat or liver)	M4 (free and conjugated), ex-pressed as pinoxaden (EFSA, 2021	0.01 mg/kg	Common limit
Body fluids		0.01 mg/L	

(a) Residue definition proposed by applicant in ongoing Article 10 evaluation (EFSA Register of Questions number EFSA-Q-2017-00280). Provisional residue definition for monitoring in plant matrices according to the EU approval of pinoxaden (EFSA, 2013b): *Sum of M4 and M6 expressed as parent pinoxaden (to include free and conjugated residues of M4 and M6).* Current residue definition (~~Reg. 839/2008~~ Reg. (EU) 2022/1346): ~~*Pinoxaden*~~ *Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden*
** indicates lower limit of analytical determination.*

zRMS comments:
According to the EFSA Journal 2021;19(3):6503 the peer review set the residue definition for risk assessment for cereals as sum of M4 and M6 (both free and conjugated), expressed as pinoxaden.
EFSA presented two options for the enforcement residue definition for further consideration by risk managers.
RD-Mo option 1: sum of M4 and M6 (both free and conjugated), expressed as pinoxaden;
and RD-Mo option 2: sum of M4 and M6 (both free only), expressed as pinoxaden.
Both options are restricted to cereals.
It is also noted that the residue definition for enforcement set in Regulation (EC) No 396/2005 is sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346).
EFSA (2021) concluded that *A single-residue analytical method for the enforcement of the RD-Mo option 1 at the combined LOQ of 0.03 mg/kg in dry commodities and 0.05 mg/kg in high water content commodities is available (EFSA, 2013; Austria, 2020). A multi-residue analytical method (QuEChERS based) for the enforcement of the RD-Mo option 2 at the combined LOQ of 0.03 mg/kg in all four main plant matrices is available (EFSA, 2013; Austria, 2020). According to the EURLs, the combined LOQ of 0.03 mg/kg is achievable for RD-Mo option 2 by using a QuEChERS based method in routine analyses (EURLs, 2020). During MSs consultation, EURLs proposed that the combined LOQ of 0.03 mg/kg is also achievable for RD-Mo option 1 (EFSA, 2021). EURLs informed EFSA about the commercial availability of the analytical standard for parent pinoxaden. However, metabolites M4 and M6 are not commercially available (EFSA, 2021).*

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

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

Table 5.3-11: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition:(1) ~~Pinoxaden (Reg. (EC) No 839/2008)~~ Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346)
(2) Sum of M4 and M6 expressed as parent pinoxaden (to include free and conjugated residues of M4 and M6); provisionally
M6 (free metabolite) only has been proposed as enforcement residue definition for plant products (cereals). However, the peer review did not come to a final agreement (EFSA, 2013)
(3) Cereal crop group (option 1): sum of M4 and M6 (both free and conjugated), expressed as pinoxaden
Cereal crop group (option 2): sum of M4 and M6 (both free only), expressed as pinoxaden (EFSA, 2021)

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High starch content (dry) *Wheat and barley grain* High water content *Wheat and barley whole plant* ~~No group~~ Dry commodities *Wheat straw*	Primary Method REM 199.03 Metabolites NOA407854 (M2), SYN 505164 (M4), SYN 502836 (M6), SYN 505887 (M 10)	0.01 mg/kg for grain 0.02 mg/kg for whole plant 0.02 mg/kg for straw	LC-MS/MS Off line SPE, extraction by relfux with HCl	Crook S.J., 2004 Report no: REM 199.03 Validation: Anderson, L., 2003 Report No.: 3030/01 *EU agreed (DAR Volume 3, Annex B, B.1 – B.5, July 2006)*
High water content	ILV	0.01 mg/kg for	LC-MS/MS	Peatman M. and Irlam S., 2003

Component of residue definition:(1) ~~Pinoxaden (Reg. (EC) No 839/2008)~~ **Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346)** **(2) Sum of M4 and M6 expressed as parent pinoxaden (to include free and conjugated residues of M4 and M6); provisionally** **M6 (free metabolite) only has been proposed as enforcement residue definition for plant products (cereals). However, the peer review did not come to a final agreement (EFSA, 2013)** **(3) Cereal crop group (option 1): sum of M4 and M6 (both free and conjugated), expressed as pinoxaden** **Cereal crop group (option 2): sum of M4 and M6 (both free only), expressed as pinoxaden (EFSA, 2021)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat and barley whole plant ~~No group~~ Dry commodities *Wheat straw*	Metabolites NOA407854 (M2), SYN 505164 (M4), SYN 502836 (M6), SYN 505887 (M 10)	grain 0.02 mg/kg for whole plant		Report no: 1983/060-D2149 *EU agreed (DAR Volume 3, Annex B, B.1 – B.5, July 2006)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
High water content *-lettuce (head)*	Primary free M4 and M6	0.01 mg/kg	LC-MS/MS QuEChERS method	Amic S., 2012 Report no: S12-04302 *EU agreed (Final Addendum to the DAR, 2013 – Addendum 3 and EFSA Journal 2013;11(8):3269)*
High acid content *- Oranges (whole fruit*)				
High oil content *- Oilseed rape (seed)*				
High protein/high starch content (dry) *- barley (grain)*				
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
High water content *-lettuce*	ILV M4 and M6 Free forms of metabolites M4 and M6	0.01 mg/kg	LC–MS/MS QuEChERS method	*EU agreed (EFSA, 2013; Austria, 2020; EURLs, 2020)* ILV Richter, S, 2015 Report No. P 3516 G New data - EU evaluated associated with the Art 12. (EFSA Journal 2021; 19 (3): 6503)*
High protein/high starch content (dry) *-wheat grain*				
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

*Evaluation report by Austria (2020) included

No new data are presented in Appendix 2.

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Hamlet J., Crook S. and Benner J., 2003, Report no: RJ3408B The extraction efficiency of methods REM 199.02 and REM 199.03 for determining residues of pinoxaden in cereals samples was compared to extraction procedures (Syngenta Method 117-01) used in crop metabolism studies in cereals. The results for the quantitative analysis of the four metabolites (NOA407854 (M2), SYN505164 (M4), SYN502836 (M6), and SYN505887 (M10)) in mature commodities, grain, straw and husks demonstrated satisfactory agreement between LC-MS/MS and the radiochemical methods, when the hydrolysis method is compared (analytical method versus „with hydrolysis approach" in the metabolism study). The metabolism study had employed longer times of acid reflux (1M HCL for 6 hours) than the residue analytical methods (reflux using 1N HCL for 2 hours). This shows that use of the analytical methods REM 199.02 and

	Method for products of plant origin
	REM 199.03 seems to be a suitable approach for assessing residues of interest for consumer exposure. *EU agreed (Final Addendum to the DAR, 2013 – Addendum 2, January 2012)*
Not required, because:	-

zRMS comments:
According to the EFSA Journal 2021;19(3):6503:
In the framework of the peer review (United Kingdom 2005; EFSA, 2013), a single residue method based on liquid chromatography with tandem mass spectrometry (LC–MS/MS) involving a hydrolysis step for the determination of free and conjugated forms of metabolites M4 and M6 was validated for dry commodities (wheat and barley grain), high water content commodities (wheat and barley whole plant) and matrices difficult to analyse (wheat straw) with an limit of quantification (LOQ) of 0.01 mg/kg for each metabolite in dry commodities, and 0.02 mg/kg for each metabolite in high water content and cereal straw. For completeness, the method was also validated for M2 and M10. The independent laboratory validation (ILV) was available, but no confirmation method, which was identified as data gap in the EFSA conclusion (EFSA, 2013). To address this data gap, an update of the LC–MS/MS analytical method including a second transition was submitted under this review (Austria, 2020). The confirmation method was validated for the determination of free and conjugated forms of M4 and M6 in high water content (lettuce), high acid content (orange), high oil content (oilseed rape) and dry commodities (barley grain, lentils), as well as in matrices difficult to analyse (wheat straw), with LOQ of 0.01 mg/kg for each metabolite. EFSA considers that the data gap set in the conclusion for the confirmatory method is addressed.
The free forms of metabolites M4 and M6 can be determined by multiresidue QuEChERS based LC–MS/MS in high water content (lettuce), high acid content (orange), high oil content (rape seed) and dry commodities (barley grain) with an LOQ of 0.01 mg/kg for each metabolite (United Kingdom, 2013). At the time of the peer review, the ILV was not available (EFSA, 2013). An ILV of the QuEChERS method on high water content (lettuce) and dry commodities (wheat grain) has been submitted in the framework of this MRL review (Austria, 2020) and it is considered sufficient for the four main matrix groups.

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

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

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

Component of residue definition:
(1) ~~Pinoxaden (Reg. (EC) No 839/2008)~~ Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346)
(2) None necessary as a result of the representative use; however M4 would be the most suitable component for ruminant matrices based on exposure resulting from the representative use in cereals (EFSA, 2013)
(3) M4 (free and conjugated), expressed as pinoxaden (EFSA, 2021)

Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Cattle muscle, kidney, fat, liver and milk Chicken liver, muscle, fat and eggs	Primary method T001530-03 M4 and M6	0.01 mg/kg for milk 0.02 mg/kg for animal tissues and eggs	HPLC MS/MS	Lin K., 2003 Report no: T001530-03 *EU agreed (Final Addendum to the DAR, 2013 – Addendum 2, January 2012 and EFSA Journal 2013;11(8):3269)*
Muscle, kidney, liver, fat, milk and eggs	Primary method T001530-03 M4 and M6	0.01 mg/kg for milk 0.02 mg/kg for liver, kidney, muscle, fat and eggs	LC/LC-MS/MS	Homazava, N., 2020 Report no: TK0529647 SYN (ADAMA has LoA) New data

Component of residue definition:
(1) ~~Pinoxaden (Reg. (EC) No 839/2008)~~ Sum of M4 and M6 (both free and conjugated), expressed as pinoxaden (Reg. (EU) 2022/1346)
(2) None necessary as a result of the representative use; however M4 would be the most suitable component for ruminant matrices based on exposure resulting from the representative use in cereals (EFSA, 2013)
(3) M4 (free and conjugated), expressed as pinoxaden (EFSA, 2021)

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC/LC-MS/MS)	
Cattle muscle, cattle fat, eggs, milk	ILV for method T001530-03 M4 and M6	0.02 mg/kg cattle muscle, fat 0.01 mg/kg for milk	HPLC MS/MS	Faltynski, K. 2003 Report no: 1467-03 *EU agreed (Final Addendum to the DAR, 2013 – Addendum 2, January 2012 and EFSA Journal 2013;11(8):3269)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	

No new data are presented in Appendix 2.

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Based on plant metabolism data, residues of pinoxaden are not anticipated to be above the LOQ in any matrix. However, it should be noted that the extraction solvent used in analytical method T001530-03 is comparable to that used in the crop monitoring method REM 199.03, for which extraction efficiency has been demonstrated: Extraction Efficiency Report: Hamlet, J., Crook, S. and Benner, J. (2003) NOA407855 - Assessment of the Efficiency of Extraction of Metabolites from Cereal Samples Following Residue Methods REM 199.02 and REM 199.03 and Syngenta Method 117-01. Syngenta, Jealott's Hill International Research Centre, Bracknell, United Kingdom. Syngenta Report No: RJ3408B; Syngenta File No: NOA407855/0504 EU Agreed (United Kingdom, 2013)

zRMS comments:
According to the EFSA Journal 2021;19(3):6503:
An analytical method, involving a hydrolysis step, for the enforcement of the proposed residue definition at the LOQ of 0.01 mg/kg in milk, and 0.02 mg/kg in animal tissues and eggs is available (EFSA, 2013). A confirmatory method is still required (data gap). In case of future needs, the method could also be applied to metabolite M6 at the same LOQs, in the same matrices (confirmation also missing for M6). According to the EURLs, metabolite M4 (free only) can be monitored in milk and in liver at the LOQ of 0.01 mg/kg using a QuEChERS based method in routine analysis. Judging from the analytical behaviour of M4, an LOQ of 0.01 mg/kg is supposed to be achievable also for the other main groups of animal products (egg, muscle, kidney, fat) (EURLs, 2020). It is reiterated that the analytical standard of metabolite M4 is not commercially available.
Remark:
In Reg. (EU) 2022/1346 it is stated that *The EU reference laboratories identified the reference standard for the M5-Metabolite (M4-conjugate) as commercially not available. When reviewing the MRLs, the Commission will take into account the commercial availability of the reference standard referred to it in the first sentence by 02/08/2023, or, if that reference standard is not commercially available by that date, the unavailability of it.*
For product of animal origin: *The European Food Safety Authority identified some information on confirmatory methods as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 2 August 2024, or, if that information is not submitted by that date, the lack of it.*

During the commenting period Applicant provided additional data. Adama has access to Pinoxaden active substance data *via* LoA for the submission of the product Edaptis. Syngenta wishes to point out that those studies are active substance information that are also submitted in the ongoing AIR6 evaluation. Double evaluation of those studies should be avoided and therefore Syngenta is of the opinion that those information are not required on product level.
The details of study of Homazava, N., 2020 (Report no: TK0529647) please refer to Appendix 2.
Analytical method T001530-03 has been acceptably validated for the determination of residues of metabolites SYN505164 and SYN502836 in animal matrices (muscle, kidney, liver, fat, milk and eggs) by LC/LC-MS/MS with limit of quantification of 0.01 mg/kg for milk and 0.02 mg/kg for liver, kidney, muscle, fat and eggs.
The method is acceptable.

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

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

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

Component of residue definition: according to EFSA Journal 2013;11(8):3269 Soil: NOA 407854 (M2) and NOA 447204 (M3)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Soil (from Washington and North Dakota)	Method 35-01 Pinoxaden (NOA407855), Metabolites NOA407854 (M2) and NOA447204 (M3)	0 0005 mg/kg	LC-MS/MS	Chamkasem N., 2003 Report no: 35-01 *EU agreed (DAR Volume 3, Annex B, B.1 – B.5, July 2006)*
Loamy sand (Pappelacker soil) Silty Clay Loam soil (Scheueracker soil)	Primary and confirmatory method GRM017.05A Pinoxaden (NOA407855), Metabolites NOA407854 (M2) and NOA447204 (M3)	0.5 µg/kg, 0.0005 mg/kg	LC-MS/MS	Method: Hargreaves S.L., 2007 Validation: Nagra B.S., 2010 Report No. T008124-05 *EU agreed (Final Addendum to the DAR, 2013)*

No new data are presented in Appendix 2.

zRMS comments:
Analytical methods for monitoring of residues of pinoxaden in soil (residue definition: NOA 407854 (M2) and NOA 447204 (M3)) have previously been considered during evaluation of the active for approval.
No further consideration is required.

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

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

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

Component of residue definition: according to EFSA Journal 2013;11(8):3269 Surface water: NOA 407854 (M2) Drinking/ground water: Pinoxaden (NOA 407855), NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56

Matrix type	Method type and analyte	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Surface and drinking water	Primary and confirmatory method RAM 414/02 Pinoxaden (NOA 407855)	0.05 µg/L	LC-MS/MS	Method: Robinson N., 2003 Report no: RAM 414/02 Validation: Robinson N., 2004 Report no: RJ3381B *EU agreed, DAR Volume 3, Annex B, B.1 – B.5, July 2006*
Surface and drinking water	Primary and con-firmatory method RAM 199.01 Metabolites NOA407854 (M2) and NOA447204 (M3)	0.05 µg/L	LC-MS/MS	Method: Figueiredo J., 2001 Report no: REM 199.01 Validation: Kissling M., 2001 Report no: 329/00 *EU agreed, DAR Volume 3, Annex B, B.1 – B.5, July 2006*
River, ground and drinking water	Primary and confirmatory method GRM017.01A Pinoxaden	0.05 µg/L	LC-MS/MS	Method: Hargreaves S.L., 2006 Validation: Tummon O.J. 2006 Report No. T008126-05-REG. *EU agreed (Final Addendum to the DAR, 2013)*
River, ground and drinking water	Primary and con-firmatory method GRM017.04A Pinoxaden Metabolites NOA407854 (M2) and NOA447204 (M3)	0.05 µg/L	LC-MS/MS	Method: Hargreaves S.L., 2007 Validation: Emburey S.N., 2007 Report No. T004028-06-REG *EU agreed (Final Addendum to the DAR, 2013)*
Water	GRM017.06A Pinoxaden and its metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108	0.05 µg/L	LC-MS/MS	Method (KCP 5.2/02): Crook S., Langridge G., McCarthy I., 2015 NOA407855_10321-1 GRM017.06A TK0201316 **New Data***
Water	GRM017.06A Pinoxaden and its metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108	0.05 µg/L	LC-MS/MS	Validation (KCP 5.2/03): Langridge G., 2015 Report No.: CEMR-6750-REG, Study No.: CEMS-6750, ASB2016-2671 **New Data***
Water	Primary GRM017.06B Pinoxaden and Its Metabolites,	0.025 µg/L	LC-MS/MS	Method (KCP 5.2/04): Langridge and Crook, 2017, Report No GRM017.06B

	NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108			SYN File No VV-132772 (NOA407855_10407). **New data**
Water	Primary GRM017.06B Pinoxaden and Its Metabolites, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108	0.025 µg/L	LC-MS/MS	Validation (KCP 5.2/05): Langridge, 2017. Report No CEMR-7546-REG SYN File No VV-466642 (NOA407855_10406) **New data**
Water	ILV of method GRM017.06B Pinoxaden and its metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108	0.025 µg/L	LC-MS/MS	ILV (KCP 5.2/06): Watson G., 2017 Report No. RES-00108 Syngenta File VV-468411 **New Data***

* Syngenta submitted data on method validation of the determination of pinoxaden and its metabolites in water as confirmatory data in the EU review of pinoxaden in April 2019.
Note: NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 is identical with pinoxaden and metabolites M2, M3, M11, M52, M54, M55 and M56

zRMS comments:
Analytical methods for monitoring of residues of pinoxaden in surface water (residue definition: NOA 407854 (M2)) have previously been considered during evaluation of the active for approval.

The current residue definition for drinking/ground water is pinoxaden (NOA 407855), NOA 407854 (M2), NOA 447204 (M3), M11, M52, M54, M55, M56.
Analytical methods for drinking/ground water to determination of residues of pinoxaden, M2 and M3 have previously been assessed during evaluation of the active for approval. There is a data gap for a monitoring method for the metabolites M11, M52, M54, M55 an M56 in ground water.

The validated method for analysis of metabolites M2, M3, M11, M52, M54, M55 and M56 in water was submitted as part of the confirmatory information of pinoxaden and has been evaluated by RMS Austria. For more details please refer to Appendix 2. The methods are acceptable.
No further data is required.

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

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

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

Component of residue definition: according to EFSA Journal 2013;11(8):3269 Air: Pinoxaden (NOA 407855)			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary and confirmatory method A.13.S267	1 µg/m^3	LC-MS/MS	Method: Strebler A., 2003 Report no: A.13S267_1

Component of residue definition: according to EFSA Journal 2013;11(8):3269 Air: Pinoxaden (NOA 407855)			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Pinoxaden (NOA407855)			Validation: Köhne A, 2003 Report no: L03-004816 *EU agreed, DAR Volume 3, Annex B, B.1 – B.5, July 2006*
Primary and confirmatory method A.13.S267 (up-date)	1 µg/m^3	LC-MS/MS	Tummon O.J., 2005, Report no: RJ3588B *EU agreed (Final Addendum to the DAR, 2013 – Addendum 3 and EFSA Journal 2013;11(8):3269)*

No new data are presented in Appendix 2.

zRMS comments:
Analytical methods for monitoring of residues of pinoxaden in air with LOQ of 1 µg/m^3 (residue definition: pinoxaden (NOA 407855)) have previously been considered during evaluation of the active for approval.
No further consideration is required.

5.3.3.7 Description of methods for the analysis of pinoxaden in body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of pinoxaden in body fluids and tissues is given in the following table.

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

Component of residue definition: Pinoxaden				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Cattle kidney and liver	Primary method T001530-03 M4 and M6	0.02 mg/kg	HPLC-MS/MS	Lin K., 2003 Report no: T001530-03 *EU agreed (Final Addendum to the DAR, 2013 – Addendum 2, January 2012 and EFSA Journal 2013;11(8):3269)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
Muscle, kidney, liver, fat, milk and eggs	Primary method T001530-03 M4 and M6	0.01 mg/kg for milk 0.02 mg/kg for liver, kidney, muscle, fat and eggs	LC/LC-MS/MS	Homazava, N., 2020 Report no: TK0529647 SYN (ADAMA has LoA) New data
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC/LC-MS/MS)	
Body fluids bovine blood	Primary and confirmatory Pinoxaden (NOA407854)	0.01 mg/L for blood	LC-MS/MS	Bejan. I, 2022 Report no: S22-05825 SYN (ADAMA has LoA) **New Data***
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

* Data requirements on the active substance are addressed by Syngenta. Syngenta has informed us that: Pinoxaden is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required. This is the information included in Syngenta's applications.

zRMS comments:
According to the SANTE/2020/12830, Rev.1, 24. February 2021 analytical methods for monitoring residues in body fluids and tissues with the LOQ of 0.01 mg/L for body fluids and 0.01 mg/kg for body tissues should be validated. The applicant did not submit an available analytical method for the determination of pinoxaden in body fluids (data gap).

During the commenting period Applicant provided additional data. Adama has access to Pinoxaden active substance data *via* LoA for the submission of the product Edaptis. Syngenta wishes to point out that those studies are active substance information that are also submitted in the ongoing AIR6 evaluation. Double evaluation of those studies should be avoided and therefore Syngenta is of the opinion that those information are not required on product level.
The details of studies of Homazava, N., 2020 (Report no: TK0529647) and Bejan. I, 2022 (Report no: S22-05825) please refer to Appendix 2.

1) Analytical method T001530-03 has been acceptably validated for the determination of residues of metabolites SYN505164 and SYN502836 in animal matrices (muscle, kidney, liver, fat, milk and eggs) by LC/LC-MS/MS with limit of quantification of 0.01 mg/kg for milk and 0.02 mg/kg for liver, kidney, muscle, fat and eggs.
2) Method QuEChERS has been acceptably validated for the determination of residues of pinoxaden (NOA407854) in bovine blood with a limit of quantification (LOQ) of 0.01 mg/L. The method complies with the data requirements given in SANTE/2020/12830.

The methods are acceptable.

5.3.3.8 Other studies/ information

None.

5.3.4 Description of analytical methods for the determination of residues of mefenpyr-diethyl (KCP 5.2)

zRMS comments:
It should be noted that mefenpyr-diethyl as safener is not considered as an active substance, consequently has not been subject to review on EU level for inclusion into Annex I of Directive 91/414/EEC or Regulation (EC) No 1107/2009 and at present MRLs are not set in the EU for safeners.

The Applicant provided the data for safener, for mefenpyr-diethyl, reviewed by Austria and France in 2011, but has not been assessed at EU level. According to Regulation 1107/2009, data for safener should be evaluated in line with requirements relevant for active substances and EU agreed and peer-reviewed endpoints should be generated. Such evaluation, however, is outside the scope of the product registration and should be carried out at the EU level in order to derive uniform endpoints that may be used in evaluation of various formulations. For this reason data provided for mefenpyr-diethyl were not validated by the zRMS.

Available residue data presented in point 5.3.4 are compliant with data presented in Monograph for mefenpyr-diethyl and are considered informative.

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

Mefenpyr-diethyl is a safener used in combination with herbicides and was not reviewed under Directive 91/414/EEC. However, a Draft Assessment Report (2011), written by Austria and France is available to all Member States. Bilateral peer-review in the form of comments took place between the two rapporteurs and respective reporting tables were made available to all MS.
No MRLs for the safener have been set at European level. Residue levels in wheat after application of mefenpyr-diethyl were all below the LOQ of 0.01 mg/kg for each analyte.

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

Table 5.3-19: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition (1)	MRL / limit	Reference for MRL/level Remarks(1)
Plant, high water content	Mefenpyr-diethyl (AE F107892) and metabolite 1(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylicacid (AEF094270) expressed as Mefenpyr-diethyl	LOQ = 0.01 mg/kg	LoEP October 2011
Plant, high acid content		LOQ = 0.01 mg/kg	LoEP October 2011
Plant, high protein/high starch content (dry commodities)		LOQ = 0.01 mg/kg	LoEP October 2011
Plant, high oil content		LOQ = 0.01 mg/kg	LoEP October 2011
Plant, difficult matrices (hops, spices, tea)		No data	LoEP October 2011
Muscle	Mefenpyr-diethyl (AE F107892) and metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2pyrazoline-3-carboxylic acid (AE F113225) expressed as Mefenpyr-diethyl	LOQ = 0.01 mg/kg	LoEP October 2011
Milk		LOQ = 0.01 mg/kg	LoEP October 2011
Eggs		No data	LoEP October 2011
Fat		LOQ = 0.01 mg/kg	LoEP October 2011
Liver, kidney		LOQ = 0.01 mg/kg	LoEP October 2011
Soil (Ecotoxicology)	Mefenpyr-diethyl (AE F107892) and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid (AE F094270)	LOQ = 0.005 mg/kg Method required for metabolite AE F094270	LoEP October 2011
Drinking water (Human toxicology)	Mefenpyr-diethyl (AE F107892) and metabolite 1-	LOQ = 0.05 µg/L	LoEP October 2011

Matrix	Residue definition (1)	MRL / limit	Reference for MRL/level Remarks(1)
	(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid (AE F094270)		
Surface water (Ecotoxicology)	Mefenpyr-diethyl (AE F107892) and metabolite 1-(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2-pyrazoline-3-carboxylic acid (AE F113225)	LOQ = 0.05 µg/L	LoEP October 2011
Air	Mefenpyr-diethyl (AE F107892)	8 µg/m^3	AOEL: 0.1 mg/kg bw/s
Tissue (meat or liver)	Mefenpyr-diethyl (AE F107892) and metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2pyrazoline-3-carboxylic acid (AE F113225) ex-pressed as Mefenpyr-diethyl	LOQ = 0.01 mg/kg	LoEP October 2011
Body fluids	No data	No data	LoEP October 2011

(1) LoEP October 2011, Austria/France

5.3.4.2 Description of analytical methods for the determination of mefenpyr-diethyl residues in plant matrices (KCP 5.2)

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

Table 5.3-20: 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 (1): **Cereal grain: Mefenpyr-diethyl (AE F107892) and metabolite AE F094270 (pyrazole carboxylic acid) expressed as mefenpyr-diethyl.** **Cereal shoot and straw: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225 (pyrazoline ester carboxylic acid), AE F109453 (pyrazoline dicarboxylic acid) and AE F094270 (pyrazole carboxylic acid) expressed as mefenpyr-diethyl (Austria, 2011)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/ high starch content (dry) - W*heat and barley grain*	Primary Method 00814 Mefenpyr-diethyl and AE F094270	0.01 mg/kg	HPLC-MS/MS	Preu T., Philipowski C. (2004) Report No.: MR-042/03 *EU agreed (DAR Volume 3, Annex B.5, July 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
High water content - *Tomato* High oil content - *Rape seed* High acid content -*Orange fruit*	Primary Method 00814 Mefenpyr-diethyl and AE F094270	0.01 mg/kg	HPLC-MS/MS	Zimmer D., Kuppels U. (2004a, b) and amendment Report No.: MR-031/04 *EU agreed (DAR Volume 3, Annex B.5, July 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	

Component of residue definition (1): **Cereal grain: Mefenpyr-diethyl (AE F107892) and metabolite AE F094270 (pyrazole carboxylic acid) expressed as mefenpyr-diethyl.** **Cereal shoot and straw: Mefenpyr-diethyl (AE F107892) and metabolites AE F113225 (pyrazoline ester carboxylic acid), AE F109453 (pyrazoline dicarboxylic acid) and AE F094270 (pyrazole carboxylic acid) expressed as mefenpyr-diethyl (Austria, 2011)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High protein/ high starch content (dry) *-Cereal grain.* High water content - *Tomato* High oil content - *Rape seed* High acid content *-Orange fruit*	ILV – Method 00841 and 00814/M001 Mefenpyr-diethyl and AE F094270	0.01 mg/kg	HPLC-MS/MS	Richter M., Class Th. (2005) Study No.: P612040559 Report No. P/B 839 G *EU agreed (DAR Volume 3, Annex B.5, July 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
High protein/ high starch content (dry) *-Cereal grain.* *- White beans* High water content - *Cereal green material* High oil content - *Oilseed rape seed* High acid content *-Orange fruit*	Primary method 01300/M001	0.01 mg/kg	LC-MS/MS - QuEChERS	Jooss S. (2010) Report No.: P 2051 G *EU agreed (DAR Volume 3, Annex B.5, Addendum 1, September 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	
High protein/ high starch content (dry) *-Cereal grain.* *- White beans* High water content - *Cereal green material* High oil content - *Oilseed rape seed* High acid content *-Orange fruit*	ILV - Method 01300/M001	0.01 mg/kg	LC-MS/MS - QuEChERS	Konrad S. (2011) Report No.: 2011/0026/01 *EU agreed (DAR Volume 3, Annex B.5, Addendum 1, September 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	

(1) LoEP October 2011, Austria/France

No new data are presented in Appendix 2.

Table 5.3-21: Statement on extraction efficiency

	Method for products of plant origin
Required, available from	The extractability of mefenpyr-diethyl (AE F107892) and metabolite AE F094270 residues in grain and mefenpyr-diethyl (AE F107892) and metabolites AE F113225, AE F109453 and AE F094270 in straw has been demonstrated in radiolabelled metabolism study CM90/069 by Bürkle, W.L., 1994 (see DAR Volume 3, B7, 2011). The extraction was performed with acetone and acetone/water followed by alkalic hydrolysis. The solvent extraction results in 77.9% TRR in grain and 72.2% TRR in straw. This sample extraction procedure is comparable with the method used in residue analytical method

	Method for products of plant origin
	00814 by Preu T., Philipowski C., 2004 (see DAR Volume 3, B5, 2011). No further study is required.
Not required, because:	-

5.3.4.3 Description of analytical methods for the determination of mefenpyr-diethyl residues in animal matrices (KCP 5.2)

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

Table 5.3-22: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: **Mefenpyr-diethyl (AE F107892) and metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2pyrazoline-3-carboxylic acid (AE F113225) expressed as Mefenpyr-diethyl** **(LoEP October 2011, Austria/France)**				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Animal matrices Fat, milk, meat, kidney, liver	Primary Method 01027 Mefenpyr-diethyl, AE F113225 and AE F094270	0.01 mg/kg	LC-MS/MS	Zimmer, D., Stucke, S. (2007) Report no: MR-06/157 *EU agreed (DAR Volume 3, Annex B.5, July 2011)*
	ILV - Method 01027 Mefenpyr-diethyl, AE F113225 and AE F094270	0.01 mg/kg	LC-MS/MS	Rzepka, S., Rotzoll, N. (2007) Final Report: BAY-0701V *EU agreed (DAR Volume 3, Annex B.5, July 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-MS/MS)	

No new data are presented in Appendix 2.

Table 5.3-23: Statement on extraction efficiency

	Method for products of animal origin
Required, available from	-
Not required, because:	Residue levels in milk and eggs were very low, in general, and reached a plateau by day 3 (milk) and day 7 (eggs), respectively. The excretion was rapid in all cases (> 80% of the TRR within 24h) indicating that there is no potential for bioaccumulation. Based on low transfer factors (please see point B.7.8 Livestock feeding studies) and on the used high dose levels (administered in the livestock metabolism studies), compared to the low residues in grain and straw (up to BBCH32) detected in field trials, no residues are to be expected in food of animal origin." (Austria, 2011) Therefore, there is no need to address extraction efficiency.

No new data are presented in Appendix 2.

5.3.4.4 Description of methods for the analysis of mefenpyr-diethyl in soil (KCP 5.2)

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

Table 5.3-24: Validated methods for mefenpyr-diethyl in soil (if appropriate)

Residue definition for monitoring in soil: Mefenpyr-diethyl (AE F107892) and its metabolite 1-(2,4dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid (AE F094270) (DAR October 2011				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Soils Silt loam soil (Höfchen) Sandy loam soil (Laacher Hof)	Primary Method 00996 Mefenpyr-diethyl (AE F107892)	5 µg/kg (= 0.005 mg/kg)	LC-MS/MS	Brumhard B., Schneider U. (2007) Report no.: MR-06/071N *EU agreed (DAR Volume 3, Annex B.5, July 2011)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	
Soils Silt loam soil (Höfchen) Sandy loam soil (Laacher Hof) Clay loam soil (Dollendorf).	Primary Method 00996 Mefenpyr-diethyl (AE F107892), AE F094270 and AE F113225	5 µg/kg (= 0.005 mg/kg)	LC-MS/MS	Freitag T. (2013) Report No.: MR-12/098 *EU agreed (DAR Volume 3, Annex B.5, Addendum 2, December 2013)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (LC-MS/MS)	

No new data are presented in Appendix 2.

5.3.4.5 Description of methods for the analysis of mefenpyr-diethyl in water (KCP 5.2)

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

Table 5.3-25: Validated methods for water (if appropriate)

Component of residue definition: (DAR, 2011) **Surface water:** Mefenpyr-diethyl (AE F107892) and its metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2pyrazoline-3-carboxylic acid (AE F113225) **Drinking / ground water:** Mefenpyr-diethyl (AE F107892) and its metabolite 1(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid (AE F094270)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Surface and ground water	Primary method 01059 Mefenpyr-diethyl (AE F107892)	0.05 µg/L	LC-MS/MS	Krebber, R., Leppelt, L. (2007) Report no.: MR-07/293 *EU agreed (DAR Addendum Volume 3, Annex B.5, revision 2, 2, October 2011)*
	ILV	--	--	No data
	Confirmatory	--	Not required, highly specific detection system was used (LC-MS/MS)	
Surface water	Primary Method 01363 Mefenpyr-diethyl (AE F107892), AE F094270 and AE	5 µg/L	LC-MS/MS	Krebber R, Braune M (2013) Method No.: 01363 Report No.: MR-13/017 *EU agreed (DAR Volume 3,*

Component of residue definition: (DAR, 2011) **Surface water:** Mefenpyr-diethyl (AE F107892) and its metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2pyrazoline-3-carboxylic acid (AE F113225) **Drinking / ground water:** Mefenpyr-diethyl (AE F107892) and its metabolite 1(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid (AE F094270)				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	F113225			*Annex B.5, Addendum 2, December 2013)*
	Confirmatory	--	Not required, highly specific detection system was used (LC-MS/MS)	

No new data are presented in Appendix 2.

5.3.4.6 Description of methods for the analysis of mefenpyr-diethyl in air (KCP 5.2)

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

Table 5.3-26: Validated methods for air (if appropriate)

Component of residue definition: Mefenpyr-diethyl (AE F107892) (DAR October 2011)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	8 µg/m^3	HPLC-UV	Method: Mutzel M., Class T. (1994) Report No.: AL002/94-0 Validation: Class T. (1994) Report no.: B141G *EU agreed (DAR Addendum Volume 3, Annex B.5, revision 2, 2, October 2011)*
Confirmatory (if required)	--	Not required, specific detection system was used (HPLC-UV)	

No new data are presented in Appendix 2.

5.3.4.7 Description of methods for the analysis of Mefenpyr-diethyl in body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of mefenpyr-diethyl in body fluids and tissues is given in the following table.

Table 5.3-27: Methods for body fluids and tissues (if appropriate)

Component of residue definition: **For animal tissues: Mefenpyr-diethyl (AE F107892) and metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2-pyrazoline-3-carboxylic acid (AE F113225) expressed as Mefenpyr-diethyl** **No residue definition published for blood and urine**				
Matrix type	**Method type, Analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Kidney, liver	Primary Method 01027 Mefenpyr-diethyl, AE F113225 and AE F094270	0.01 mg/kg	LC-MS/MS	Zinmer, D., Stucke, S. (2007) Report no: MR-06/157 *EU agreed (DAR Addendum Volume 3, Annex B.5, revision 2, 2, October 2011)*

Component of residue definition: **For animal tissues: Mefenpyr-diethyl (AE F107892) and metabolite 1(2,4-dichlorophenyl)-5-ethoxycarbonyl-5-methyl-2-pyrazoline-3-carboxylic acid (AE F113225) expressed as Mefenpyr-diethyl** **No residue definition published for blood and urine**				
Matrix type	**Method type, Analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory	--	Not required, highly specific detection system was used (LC-MS/MS)	

No new data are presented in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Tsesin N.	2020	Determination of Storage Stability and Physical-Chemical Properties of Mesosulfuron-methyl 12 g/l + Pinoxaden 60 g/l + Mefenpyr-diethyl 35 g/l OD (ADM.06001.H.2.B) Stored at 54°C for 14 Days and at 0°C for 7 Days Report no. 000105084.069FL, ADAMA reference no. 000105084 ADAMA Makhteshim Ltd., Israel GLP, unpublished Also filed under KCP 2.1/01	N	ADAMA
KCP 5.1.1/02	Ricau H.	2020	Validation of the analytical method for determination of toluene in MESOSULFURON-METHYL 12 G/L + PINOXADEN 60 G/L + MEFENPYR-DIETHYL 35 G/L OD (ADM.06001.H.2.B) Report no. 20-901066-037, ADAMA reference no. 000106124) ANADIAG Group DEFITRACES, France GLP, unpublished	N	ADAMA
KCP 5.1.2/01	Barbier G.	2019	Validation of an analytical method for the determination of mesosulfuron-methyl in wheat (whole plant, grain, straw) Report no. B19G-A4-M-03, ADAMA reference no. 000102681 FREDON Pays de la Loire / GIRPA, France GLP, unpublished	N	ADAMA
KCP 5.1.2/02	Barbier G.	2020	Validation of an analytical method for the determination of pinoxaden metabolites M4 and M6 in wheat (whole plant, grain, straw) Report no. B19G-A4-P-05, ADAMA reference no. 000102680 FREDON Pays de la Loire / GIRPA, France GLP, unpublished	N	ADAMA
KCP 5.1.2/03	Meric D.	2021	Magnitude of the residue of pinoxaden metabolites, mesosulfuron-methyl, mefenpyr-diethyl and metabolite following one application of ADM.06001.H.2.B in winter wheat in 2 trials (2 HS, one with process), Northern Europe (France and Poland) – 2020 Report no. DMC-20-42727, ADAMA reference no. 000105437 STAPHYT, 62860 Inchy en Artois, France GLP, unpublished	N	ADAMA
KCP 5.1.2/04	Lefresne S.	2019	Validation of an analytical method for the determination of mefenpyr-diethyl in wheat (whole plant, grain, straw) Report no. B19S-A4-M-01, ADAMA reference no. 000102679 FREDON Pays de la Loire / GIRPA, France GLP, unpublished	N	ADAMA

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.2/05	Seidel U. and Mollandin G.	2021a	ADM.06001.H.2.B: Acute Toxicity to *Daphnia magna* in a Semi-Static 48-hour Immobilisation Test Report no. 140711220, ADAMA reference no. 000105363 Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.2.1/01	N	ADAMA
KCP 5.1.2/06	Seidel U. and Mollandin G.	2021b	ADM.06001.H.2.B: Toxicity to *Raphidocelis subcapitata (=Pseudokirchneriella subcapitata*) in an Algal Growth Inhibition Test Report no. 140711210, ADAMA reference no. 000105364 Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.2.1/02	N	ADAMA
KCP 5.1.2/07	Seidel U. and Mollandin G.	2021c	ADM.06001.H.2.B: Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test Report no. 140711240, ADAMA reference no. 000105365 Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.2.1/03	N	ADAMA
KCP 5.1.2/08	Sekine T. and Kowalczyk F.	2021	ADM.06001.H.2.B: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera L*.) in the Laboratory Report no. 140711136, ADAMA reference no. 000105367 Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.3.1.2/01	N	ADAMA
KCP 5.1.2/09	Colli M.	2020	Effects of ADM.06001.H.2.B on honeybees (*Apis mellifera L*.) 22-day larval toxicity test with repeated exposure Report no. BT138/20, ADAMA reference no. 000105368 BioTecnologie BT S.r.l., Todi (PG), Italy GLP, unpublished Also filed under KCP 10.3.1.3/01	N	ADAMA
KCP 5.1.2/10	Straube D. and Gourlay V.	2021	ADM.06001.H.2.B: Determination of chronic toxicity to the earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae) in an artificial soil substrate Report no. 140711022, ADAMA reference no. 000105375 Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.4.1.1/01	N	ADAMA
KCP 5.1.2/11	Spatz B. and Kowalczk	2021a	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test Report no. 140711086, ADAMA reference no. 000105379	N	ADAMA

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
			Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.6.2/01		
KCP 5.1.2/12	Spatz B. and Kowalczk	2021b	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Report no. 140711087, ADAMA reference no. 000105380 Ibacon GmbH, Rossdorf, Germany GLP, unpublished Also filed under KCP 10.6.2/02	N	ADAMA
KCP 5.2/01	Watson G.	2021	Validation of an Analytical Method for the Determination of Residues of Mesosulfuron-methyl in human urine by LC-MS/MS, Final Report Amendment No.1 Report no. RES-00291, ADAMA reference no: 000106703 ResChem Analytical Limited, Derby, UK GLP, unpublished	N	ADAMA

List of data referred to by the applicant and relied on, but not 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 5.2/02	Crook S., Langridge G., McCarthy, I.	2015	Pinoxaden - Residue method GRM017.06A for the determination of Pinoxaden and its metabolites, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 in water by LCMS/MS analysis NOA407855_10321 “GRM017.06A”, TK0201316 Not GLP, unpublished	N	SYN (ADAMA has LoA)
KCP 5.2/03	Langridge G.	2015	Pinoxaden - Validation of draft residue method GRM017.06A for the determination of Pinoxaden and its metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in water, Report no: CEMR-6750-REG, Study no. CEMS-6750, ASB2016-2671 GLP, unpublished	N	SYN (ADAMA has LoA)
KCP 5.2/04	Langridge, G. Crook, S.	2017	Pinoxaden - Residue Method GRM017.06B for the Determination of Pinoxaden and Its Metabolites, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 in Water by LC-MS/MS Analysis Report No. GRM017.06B Document No. VV-132772 , NOA407855_10407 Test Facility CEM Analytical Services, Ltd. GLP	N	SYN (ADAMA has LoA)

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
KCP 5.2/05	Langridge, G.	2017	Pinoxaden – Validation of an Analytical Method for the Determination of Pinoxaden and Metabolites in Water Report No. CEMR-7546 Document No. VV-466642 , NOA407855_10406 Test Facility CEM Analytical Services, Ltd. GLP Unpublished	N	SYN (ADAMA has LoA)
KCP 5.2/~~04~~ 06	Watson G.	2017	Pinoxaden - Independent Laboratory Validation (ILV) of analytical method GRM017.06B for the determination of Pinoxaden (NOA407855) and metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 in Water. . ResChem Analytical Limited, Unit, Derby, United Kingdom. Report No. RES-00108. Syngenta File VV-468411 GLP, unpublished	N	SYN (ADAMA has LoA)
~~KCP 5.2~~	~~Richter S.~~	~~2015~~	~~Pinoxaden – Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of Pinoxaden Metabolites M4 (SYN505164) and M6 (SYN502836) in Crop Matrices by LC-MS/MS. PTRL Europe GmbH, Germany.~~ ~~Report No. P 3516 G~~ ~~Syngenta File No. SYN505164_10001~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN (ADAMA has LoA)~~
KCP 5.2/07	Homazava, N.	2020	Pinoxaden (NOA407855) - Validation of Analytical Method T001530-03 for the Determination of Residues of Metabolites SYN505164 and SYN502836 in Animal Matrices by LC/LC-MS/MS, Report number 20190507 (TK0529647); VV-872393 GLP, unpublished	N	SYN (ADAMA has LoA)
KCP 5.2/08	Bejan, I.	2022	Pinoxaden: Validation of Analytical Method QuEChERS for the Determination of Residues of NOA407854 in Body Fluid (Blood only) by LCMS/MS, Report number S22-05825 VV-967942 GLP, unpublished	N	SYN (ADAMA has LoA)

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	Previous evaluation
Mesosulfuron-methyl						
KCP 5.1.2 and KCP 5.2	Wrede A.	1999	Data generation method and validation for cereal by LC-MS/MS Code: AE F130060 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany, Bayer Crop Science Report No.:C005129, Edition Number: M-191437-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001
KCP 5.2	Wrede A.	2000	Enforcement Method for Cereal Grain, Straw and Shoot by LC-MS/MS Amidosulfuron (AE F075032) Metsulfuron-methyl (AE F075736) Iodosulfuronmethyl-sodium (AE F115008) AE F130060, AE F130360 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Bayer Crop Science Report No.: C006734, Edition Number: M-194528-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/01
KCP 5.2	Wrede A.	2000	Validation of the Enforcement Method EM F08/99-0 of cereal grain, straw and shoot by LC-MS/MS – Amidosulfuron (AE F075032) – Metsulfuron-methyl (AE F075736) Iodosulfuronmethyl-sodium (AE F115008) - AE F130060 - AE F 130360 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany, Bayer Crop Science Report No.: C006735, Edition Number: M-194531-01-1 GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/02
KCP 5.2	Wrede A.	2000	Validation of the enforcement method EM F08/99-0 in cereal grain, straw and shoot by LC-MS/MS - Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany, Bayer Crop Science Report No.: C008827, Edition Number: M-197830-01-1 GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/03
KCP 5.2	Reichert N.	2000	Independent laboratory validation of the method of analysis EM F08/99-0 for the determination of AE F130060 in cereal (grain) Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Bayer Crop Science Report No.: C009586, Edition Number: M-198857-01-1 GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/04
KCP 5.2	Wrede A.	2000	Enforcement method and validation for cereal grain by HPLC-UV Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer Crop Science Report No.: C009496, Edition Number: M-198685-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/05
KCP 5.2	Wrede A.	2000	Multi-residue method for the determination of AE F130060 in cereal grain (statement) Mesosulfuron	N	Bayer Crop	Original Annex

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			Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer Bayer Crop Science Report No.: C009649, Edition Number: M-198985-01-1 Not GLP, unpublished		Science	II submission DAR 2001, KCA 4.2/06
KCP 5.2	Wrede A.	2002	Validation of the enforcement method EM F08/99-0 for lemon, tomato and maize kernel by LC-MS/MS - Amidosulfuron (AE F075032) - Iodosulfuronmethyl-sodium (AE F115008) – Mesosulfuron-methyl (AE F130060) – Foramsulfuron (AE F130360) Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer Crop Science Report No.: C022220, Edition Number: M-212674-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/15
KCP 5.2	Reicher N.	2001	Independent laboratory validation of the method of analysis EM F08/99-0 for the determination of AE F130060 in cereal (plant and straw) Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Bayer Crop Science Report No.: C011938, Edition Number: M-201813-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/17
KCP 5.2	Reichert N., Klimmek S.	2002	Independent laboratory validation of the analytical method EM F08/99-0 for the residue analysis of Amidosulfuron (AE F075032), Iodosulfuronmethyl-sodium (AE F115008), Mesosulfuronmethyl (AE F130060), Foramsulfuron (AE F130360) in tomato and citrus Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Bayer CropScience, Report No.: C023679, Edition Number: M-215456-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/18
KCP 5.2	Heinemann O.	2004	Modification M001 to method 00815 for the determination of residues of amidosulfuron, iodosulfuronmethyl-sodium including metabolite metsulfuron-methyl, foramsulfuron and mesosulfuronmethyl in/on flax and wheat matrices by HPLC-MS/MS Bayer CropScience, Report No.:00815/M001, Edition Number: M-226888-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/16
KCP 5.2	Stuke S., Ballmann C.	2013	Analytical method 01360 for the determination of amidosulfuron, metsulfuronmethyl, iodosulfuronmethyl-sodium, mesosulfuronmethyl, and foramsulfuron in samples from plant origin by HPLC-MS/MS Bayer CropScience, Report No.: MR13/007, Edition Number: M-455564-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/19
KCP 5.2	Konrad S.	2013	Independent lab validation of BCS method 01360 for the determination of residues of amidosulfuron, metsulfuronmethyl, iodosulfuronmethyl-sodium, mesosulfuronmethyl and foramsulfuron in samples	N	Bayer Crop Science	Submitted for the purpose of

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**	**Previous evaluation**
			from plant origin by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2013/0060/01, Edition Number: M-470160-01-1 GLP, unpublished			renewal KCA 4.2/20
KCP 5.2	Stuke S.	2015	Modification 001 of analytical method 01360 for the determination of amidosulfuron, metsulfuronmethyl, iodosulfuronmethyl-sodium, mesosulfuronmethyl, and foramsulfuron in samples from plant origin by HPLC-MS/MS Bayer CropScience, Report No.: MR-15/090, Edition Number: M-537921-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/26
KCP 5.2	Schmeer K., Philipowski C.	2010	Modification M001 of the residue analytical method 01208 for the determination of amidosulfuron (AE F075032), metsulfuronmethyl (AE F075736), iodosulfuronmethyl-sodium (AE F115008), mesosulfuronmethyl (AEF130060), foramsulfuron (AE F130360) in animal tissues (meat, fat, liver, kidney), egg, and milk by HPLCMS/MS Bayer CropScience, Report No.: 01208/M001, Edition Number: M-389788-03-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/28
KCP 5.2	Netzband D.	2010	Independent laboratory validation of an analytical method 01208/M001 for the determination of amidosulfuron (AE F075032), metsulfuronmethyl (AE F075736), iodosulfuronmethyl-sodium (AE F115008), mesosulfuronmethyl (AE F130060), foramsulfuron (AE F130360) in animal tissues (meat, fat, liver, kidney), egg, and milk by HPLC-MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RAMML014-01, Edition Number: M-398300-02-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/27
KCP 5.2	Wrede A.	2000	Enforcement method and validation of soil by HPLC-UV Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C009151, Edition Number: M-198143-01-1 GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/07
KCP 5.2	Wrede A.	2000	Data generation method for soil by LC-MS/MS Amidosulfuron, metsulfuronmethyl, iodosulfuronmethyl-sodium, AE F130060, AE F130360 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C008681, Edition Number: M-197688-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.1.2/02
KCP 5.2	Wrede A.	2000	Validation of the method DGM F04/99-0 in soil by LC-MS/MS - Amidosulfuron, metsulfuronmethyl, iodosulfuronmethyl-sodium, AE F130060, AE F130360 Aventis CropScience GmbH, Frankfurt am Main, Germany	N	Bayer Crop Science	Original Annex II submission DAR 2001,

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**	**Previous evaluation**
			Bayer CropScience, Report No.: C008682, Edition Number: M-197689-01-1 GLP, unpublished			KCA 4.1.2/03
KCP 5.2	Wrede A.	2000	Enforcement Method for Soil by LC-MS/MS Metsulfuronmethyl (AE F075736) Iodosulfuronmethyl-sodium (AE F115008) Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C006394, Edition Number: M-193807-01-1 EPA MRID No.: 45108502 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/08
KCP 5.2	Wrede A.	2000	Validation of the enforcement method EM F13/99-0 of soil by LC-MS/MS - Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C009563, Edition Number: M-198810-01-1 GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/09
KCP 5.2	Freitag T.	2008	Amendment no0001 to reportno.: MR-08/138- AnalyticalMethod 01115for the determination ofresidues of amidosulfuron,iodosulfuronmethyl-sodium, metsulfuronmethyl, mesosulfuronmethyl and foramsulfuron insoil by HPLCMS/MS Bayer CropScience, Report No.: M310074-03-1, Edition Number:M-310074-03-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/21
KCP 5.2	Wrede A.	2000	Enforcement method for surface and drinking water by HPLC-UV Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C008689, Edition Number: M-197696-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/10
KCP 5.2	Wrede A.	2000	Validation of the enforcement method EM F15/99-0 for surface and drinking water by HPLC-UV - Code: AE F130060 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C008686, Edition Number: M-197693-01-1 GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/11
KCP 5.2	Wrede A.	2001	Enforcement method for surface and drinking water by LC-MS/MS – Amidosulfuron (AE F075032) Metsulfuronmethyl (AE F075736) lodosulfuronmethyl-sodium (AE F115008) Mesosulfuronmethyl (AE F130060) Foramsulfuron (AE F130360) Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C011206, Edition Number: M-200404-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.2/13
KCP 5.2	Wrede A., Neuss B.	2001	Validation of the enforcement method EMF04/00-0 for surface and drinking water by LC-MS/MS – Amidosulfuron (AE 075032) Metsulfuron-methyl (AE F075736) lodosulfuron- methyl-sodium (AE	N	Bayer Crop Science	Original Annex II submission

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	Previous evaluation
			F115008) Mesosulfuron-methyl (AE F130060) Foramsulfuron (AE F130360) Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C011207, Edition Number:M-200406-01-1 GLP, unpublished			DAR 2001, KCA 4.2/14
KCP 5.2	Krebber R., Braune, M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report No.: MR13/085, Edition Number: M-466732-01-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/22
KCP 5.2	Stanislowski T.	2013	Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS PTRL Europe, Ulm, Germany Bayer CropScience, Report No.: P3117 G, Edition Number: M-470714-02-1 GLP, unpublished	N	Bayer Crop Science	Submitted for the purpose of renewal KCA 4.2/23
KCP 5.2	Reichert N.	2000	Development and validation of an analytical method for the determination of AE F130060 in air Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Bayer CropScience, Report No.:C009587, Edition Number: M-198860-01-1 Not GLP, unpublished	N	Bayer Crop Science	Original Annex II submission DAR 2001, KCA 4.1.2/04
Pinoxaden						
KCP 5.1.2	Gasser A.	2002a	Determination of NOA 407855, SYN 505164, SYN 502836, SYN 505887 (Metabolites of NOA 407855) and CGA 153433 (Metabolite of CGA 185072) in Cereals by LC/LC-MS/MS (Validated) Syngenta Crop Protection AG, Basel, Switzerland, Report No REM 199.02 Not GLP, Not Published Syngenta File N° NOA407855/0057	N	SYN	DAR 2006, KIIA 4.2.1/01
KCP 5.1.2	Gasser A.	2002b	Validation of Method REM 199.02: Validation by Analysis of Wheat Specimens (whole plant, Straw and grains) fortified with NOA 407855, SYN 505164, SYN 502836, SYN 505887 and CGA 153433 and Determination of Recoveries Syngenta Crop Protection AG, Basel, Switzerland, Report No 02-S302 GLP, Not Published Syngenta File N° NOA407855/0058	N	SYN	DAR 2006, KIIA 4.2.1/05
KCP 5.2	Crook S.J.	2004	Residue Method for the Determination of Residues of NOA 407854, SYN 505164, SYN 502836, SYN 505887 (Metabolites of NOA 407855) and CGA 153433 (Metabolite of CGA 185072) in Cereal Samples, and Cereal Process Fractions. Final Determination by LC-MS/MS. Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill International, Bracknell, Berkshire, UK,	N	SYN	DAR 2006, KIIA 4.2.1/02

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	Previous evaluation
			Report No REM 199.03 Not GLP, Not Published Syngenta File N° NOA407855/0457			
KCP 5.2	Peatman M. and Irlam S.	2003	NOA 407854, SYN 505164, SYN 502836, SYN 505887 and CGA 153433: Independent Laboratory Validation of REM 199.03 Analytical Method for the Determination of Residues in Cereal Whole Plant and Grain Syngenta, Jealott's Hill, United Kingdom Covance Laboratories, North Yorkshire, UK, Report No 1983/060- D2149 GLP, Not Published Syngenta File N° NOA407854/0036	N	SYN	DAR 2006, KIIA 4.2.1/07
KCP 5.2	Amic S.	2012	Pinoxaden - Validation of the QuEChERS Method for the Determination of Residues of Pinoxaden Metabolites M4 (SYN505164) and M6 (SYN502836) in Crops Matrices by LC-MS/MS Syngenta Eurofins Agroscience Services Chem SAS, Vergèze, France, S12-04302 GLP, not published Syngenta File No SYN505164_10000	N	SYN	DAR Final Addendeum 2013, KIIA 4.2.1/09 (KIIA 4.3/01)
KCP 5.2	Hamlet J., Crook S. and Benner J	2003	NOA 407855: Assessment of the Efficiency of Extraction of Metabolites from Cereal Samples Following Residue Methods REM 199.02 and REM 199.03 and Syngenta Method 117-01. Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Jealott's Hill, United Kingdom, Report No RJ3408B GLP, Not Published Syngenta File N° NOA407854/0042	N	SYN	DAR 2006, KIIA 4.2.1/04
KCP 5.2	Lin K.	2003	Analytical Method for Determination of NOA 407855 Metabolites, SYN 505164 (M4) and SYN 502836 (M6) in Animal Tissues, Milk and Eggs by LC/MS/MS Including Validation Data Syngenta Crop Protection, Inc., Greensboro, United States, Report No T001530-03 GLP, Not Published Syngenta File N° NOA407855/0261	N	SYN	DAR 2006, KIIA 4.2.1/03
KCP 5.2	Faltynski K.	2003	Independent Laboratory Validation of Syngenta Method T001530-03, Analytical Method for Determination of NOA 407855 Metabolites, SYN 505164 (M4) and SYN 502836 (M6) in Animal Tissues, Milk and Eggs by LC/MS/MS Including Validation Data, On Beef Muscle Syngenta Crop Protection, Inc., Greensboro, United States, Report No 1467-03 GLP, Not Published Syngenta File N° NOA407855/0502	N	SYN	DAR 2006, KIIA 4.2.1/08

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	Previous evaluation
KCP 5.2	Chamkasem N.	2003	Analytical Method 35-01 for the Determination of NOA407855 and Its Degradates NOA407854 and NOA447204 and CGA185072 (Safener) and Its Degradate CGA153433 in Soil by High Performance Chromatography with Mass Spectrometric Detection Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, Inc., Greensboro, United States, Report No 35- 01 GLP, Not Published Syngenta File N° NOA407855/0322	N	SYN	DAR 2006, KIIA 4.2.2/01
KCP 5.2	Hargreaves S.L.	2007	Pinoxaden - Residue Method for the Determination of Pinoxaden (NOA407855) and its Metabolites NOA407854 and NOA447204 in Soil Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott˝s Hill International, Bracknell, Berkshire, United Kingdom, Report no GRM 017.05A Not GLP, not published Syngenta File No NOA407855/1033	N	SYN	DAR Final Addendeum 2013, KIIA 4.2.2/02 (KIIA 4.4/01)
KCP 5.2	Nagra B.S.	2010	Pinoxaden (NOA407855) - Validation of a Residue Method (GRM017.05A) for the Determination of Residues of Pinoxaden and its Metabolites NOA407854 and NOA447204 in Soil Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott˝s Hill International, Bracknell, Berkshire, United Kingdom, Report No T008124-05-REG GLP, not published Syngenta File No NOA407855/1032	N	SYN	DAR Final Addendeum 2013, KIIA 4.2.2/03 (KIIA 4.4/02)
KCP 5.2	Robinson N.	2003	Residue Analytical Method for the Determination of Residues of NOA407855 in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Jealott's Hill, United Kingdom, Report No RAM 414/02 GLP, Not Published Syngenta File N° NOA407855/0520	N	SYN	DAR 2006, KIIA 4.2.3/01
KCP 5.2	Robinson N.	2004	Validation of an Analytical Method for the Determination of Residues in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom, Report No RJ3381B GLP, Not Published Syngenta File N° NOA407855/0140	N	SYN	DAR 2006, KIIA 4.2.3/03
KCP 5.2	Figueiredo J.N.	2001	Determination of Metabolites NOA 407854 and NOA 447204 by LC/LC-ESI/MS/MS-MS, water	N	SYN	DAR 2006, KIIA

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	Previous evaluation
			Syngenta Crop Protection AG, Basel, Switzerland, Report No REM 199.01 GLP, Not Published Syngenta File N° NOA407855/0044			4.2.3/02
KCP 5.2	Kissling M.	2001	Validation of Method REM 199.01: Validation by Analysis of Specimens Fortified with NOA 407854 and NOA 447204 and Determination of Recoveries Syngenta Crop Protection AG, Basel, Switzerland, Report No 329/00 GLP, Not Published Syngenta File N° NOA407855/0045	N	SYN	DAR 2006, KIIA 4.2.3/04
KCP 5.2	Hargreaves S.L.	2006	Pinoxaden: Residue Method for the Determination of Residues in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott˝s Hill International, Bracknell, Berkshire, United Kingdom Report No GRM 017.01A GLP, not published Syngenta File No NOA407855/0986	N	SYN	DAR Final Addendum 2013, KIIA 4.2.3/03 (KIIA 4.5/01)
KCP 5.2	Tummon O.J.	2006	Pinoxaden (NOA407855): Validation an Analytical Method for the Determination of Residues in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott˝s Hill International, Bracknell, Berkshire, United Kingdom Report No T008126-05-REG GLP, not published Syngenta File No NOA407855/0987	N	SYN	DAR Final Addendum 2013, KIIA 4.2.3/07 (KIIA 4.5/03)
KCP 5.2	Hargreaves S.L.	2007	Pinoxaden - Residue Method for the Determination of Metabolites NOA407854 and NOA447204 and CGA185072 (Safener) and Its Metabolite CGA153433 in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott˝s Hill International, Bracknell, Berkshire, United Kingdom, Report no. GRM 017.04A GLP, not published Syngenta File No NOA407854/0062	N	SYN	DAR Final Addendum 2013, KIIA 4.2.3/04 (KIIA 4.5/02
KCP 5.2	Emburey S.N.	2007	Pinoxaden - Validation of an Analytical Method for the Determination of Residues of Its Metabolites NOA407854 and NOA447204 and the Safener CGA185072 and Its Metabolite CGA153433 in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott˝s Hill International, Bracknell, Berkshire, United Kingdom, Report No T004028-06-REG	N	SYN	DAR Final Addendum 2013, KIIA 4.2.3/08 (KIIA 4.5/04)

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**	**Previous evaluation**
			GLP, not published Syngenta File No NOA407854/0063			
KCP 5.2	Strebler A.	2003	Determination of NOA407855 in air by LC-MS/MS Syngenta Crop Protection AG, Basel, Switzerland Solvias AG, Basel, Switzerland, Report No A.13S267_1 Not GLP, Not Published Syngenta File N° NOA407855/0344	N	SYN	DAR 2006, KIIA 4.2.4/01
KCP 5.2	Köhne A	2003	Validation of the Method A.13.S267_1: Determination of NOA407855 in air by LC-MS/MS Syngenta Crop Protection AG, Basel, Switzerland Solvias AG, Basel, Switzerland, Report No L03-004816 GLP, Not Published Syngenta File N° NOA407855/0345	N	SYN	DAR 2006, KIIA 4.2.4/02
KCP 5.2	Tummon O.J.	2005	Validation of an Analytical Method for the Environmental Monitoring Determination of Pinoxaden in Air Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott‟s Hill, Bracknell, United Kingdom, Report No. RJ3588B 04-S708 GLP, not published Syngenta File No NOA407855/0750	N	SYN	DAR Final Addendum 2013, KIIA 4.2.4/03 (KIIA 4.7 / 01
Mefenpyr-diethyl						
KCP 5.2	Preu M., Philipowski C.	2004	Residue analytical method 00814 for the determination of residues of Mefenpyr-diethyl (AE F107892) and its metabolites in/on wheat and barley (green material, grain and straw) by HPLC-MS/MS Generated by Bayer CropScience AG, BCS-D-ROCS, Germany Document No: C039456 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.1/01
KCP 5.2	Zimmer D., Kuppels U.	2004a	Enforcement method 00814/M001 for the determination of residues of mefenpyr-diethyl (AEF107892) and its metabolites AE F094270 in/on plant material by HPLC-MS/MS (Method and validation) Generated by Bayer CropScience AG, Monheim, Germany Document No: C043558 GL	N	BAY	DAR 2011, IIA, 4.2.1/02

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			P, not published			
KCP 5.2	Zimmer D., Kuppels U.	2004b	Enforcement method 00814/M001 for the determination of residues of mefenpyr-diethyl (AE F107892) and its metabolites AE F094270 in/on plant material by HPLC-MS/MS, Amendment to report No. 1, dated 2004-11-25 Bayer CropScience AG, Monheim, Germany Document No: C046495 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.1/03
KCP 5.2	Richter M., Class Th.	2005	Independent laboratory validation of method 00814/M001 for the determination of residues of mefenpyr-diethyl (AE F107892) and AE F094270 in/on matrices of plant origin by HPLC-MS/MS. Demonstration of a LC/MS/MS confirmation method. PTRL Europe, Ulm, Germany Document No: C046787 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.1/04
KCP 5.2	Jooss, S.	2010	Validation of analytical BCS method01300/M001 for the determination of residues of mefenpyr-diethyl and its metabolite AE F094270 (using QuEChERS and LC/MS/MS) in/on plant materials PTRL Europe GmbH, Ulm, Germany Bayer CropScience, Method No.: 01300/M001, Method Report No.: P 2051 G GLP, not published	N	BCS	DAR Addendeum 1, 2011, IIA 4.3.9/01
KCP 5.2	Konrad, St.	2011	Validation of analytical BCS method 01300/M001 for determination of residues of mefenpyr-diethyl and its metabolite AE F094270 (using QuEChERS and LC-MS/MS) in/on plant materials Currenta GmbH & Co. OHG, D-51368 Leverkusen Bayer CropScience, Report No.: P612117508, Method Report No.: 2011/00026/01 GLP, not published	N	BCS	DAR Addendeum 1, 2011, IIA 4.3.9/02
KCP 5.2	Zimmer, D., Stuke, S.	2007	Analytical Method 01027 for the Determination of Residues of the Active Item Mefenpyr-diethyl and its Metabolites AE F113225 and AE F094270 in/on Animal Tissues and Milk by HPLC-MS/MS Bayer CropScience, Report No.: 01027 Method Report No.: MR-06/157 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.1/06
KCP 5.2	Repka, S., Rotzoll, N.	2007	Independent laboratory validation of Bayer CropScience analytical method No. 01027 for the determination of residues of Mefenpyr-diethyl and its metabolites AE F113225 and AE F094270 in/on animal tissues and milk by HPLC-MS/MS Eurofins Analytik GmbH, Hamburg, Germany	N	BAY	DAR 2011, IIA, 4.2.1/07

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	Previous evaluation
			Bayer CropScience, Report No.: BAY-0701V Method Report No.: P613065527 GLP, not published			
KCP 5.2	Brumhard, B., Schneider U.	2007	Analytical Method 00996 for the Determination of Residues of Mefenpyr-diethyl (AE F107892) in Soil by HPLC-MS/MS Bayer CropScience, Report No.: 00996 Method report No.: MR-06/071 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.2/01
KCP 5.2	Freitag Th.	2013	Modification M001 of the analytical method 00996 for the determination of mefenpyr-diethyl and the metabolites AE F094270 and AE F113225 in soil by HPLC-MS/MS Bayer CropScience AG, Study ID: P681121813 Publication ID: M-447567-01-1 GLP, not published	N	BCS	DAR Addendeum 2, 2013
KCP 5.2	Krebber, R., Leppelt, L.	2007	Analytical method 01059 for the determination of mefenpyr-diethyl in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report Number: 01059, Method report No.: 01059 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.3/01
KCP 5.2	Krebber R., Braune M.	2013	Analytical method 01363 for the determination of mefenpyr-diethyl and its metabolites AE F113225 and AE F094270 in drinking and surface water by HPLC-MS/MS Bayer CropScience AG Study ID: P 684 127077 GLP, not published	N	BCS	DAR Addendeum 2, 2013
KCP 5.2	Mutzel M., Class T.	1994	Quantitation of diethyl-1-(2,4-dichlorophenyl)-Smethyl-2-pyrazoline-3,5-dicarboxylate (Hoe 107892) in air by adsorption to Tenax and RP-HPLC/UV PTRL Europe, Labor f. Umwelt- und Pestizidchemie, Germany Document No: A53219 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.4/01
KCP 5.2	Class T.	1994	Validation of an Analytical Method for the Determination of Diethyl 1-(2,4-dichlorophenyl)-5rnethyl-2-pyrazoline-3,5-dicarboxylate in Air PTRL Europe, Labor f. Umwelt- und Pestizidchemie, Germany Document No: A52415 GLP, not published	N	BAY	DAR 2011, IIA, 4.2.4/02
KCP 5.2	Richter S.	2015	Pinoxaden – Independent Laboratory Validation of the QuEChERS Method for the Determination of	N	SYN	EFSA Journal

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	Previous evaluation
			Residues of Pinoxaden Metabolites M4 (SYN505164) and M6 (SYN502836) in Crop Matrices by LC-MS/MS. PTRL Europe GmbH, Germany. Report No. P 3516 G Syngenta File No. SYN505164_10001 GLP Unpublished		(ADAMA has LoA)	2021;19(3):6503

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for ADM.06001.H.2.B containing mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl

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

A 2.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation - Residues of mesosulfuron-methyl in plant matrices

Comments of zRMS:	The analytical method has been fully validated for the determination of mesosulfuron-methyl in wheat (whole plant, grain, straw) with the LOQ of 0.010 mg/kg in compliance with SANTE/2020/12830, Rev.1, 24. February 2021. The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than or equal to 20%. It should be noted that residues of mesosulfuron-methyl were extracted with acetonitrile/0.02 M triethylamine (80/20 v/v), the same solvents as in metabolism studies. The method is acceptable.

Reference:	KCP 5.1.2/01
Report	Validation of an analytical method for the determination of mesosulfuron-methyl in wheat (whole plant, grain, straw) Barbier G., 2019 Report no: B19G-A4-M-03, ADAMA reference no. 000102681
Guideline(s):	Yes, SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was validated for the determination of mesosulfuron-methyl residues in wheat (whole plant, grain, straw). Residues of mesosulfuron-methyl were extracted from homogenised wheat by maceration in an acetonitrile/0.02 M triethylamine (80/20 v/v) mixture. Then extracts were purified by solid/liquid partitions. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). A LOQ of 0.01 mg/kg was validated for each matrix tested.

Test substance	CAS No.	Batch / Lot No.	Purity / content
Mesosulfuron-methyl	208465-21-8	232-3283	99.6%

Sample preparation

2 g of ground sample for wheat whole plant and wheat straw or 5 g for wheat grain was weighed and transferred into a 50 mL centrifuge tube. For recoveries, the sample was fortified with the appropriate spiking standard solutions. To each sample 20 mL of an acetonitrile / 0.02 M triethylamine (80/20 v,v) mixture was added. After homogenisation one sachet of MgSO4/NaCl/salts tampon was added to the centrifuge tube. The sample was shaken and centrifuged and the final volume was 16 mL. The supernatant was analysed using HPLC-MS/MS.

Standard solutions, Fortification solutions, Matrix-matched calibration solutions:
Standard solutions at 0.1, 1 and 10 mg/L were prepared by dilution of the stock standard solution in acetonitrile. These diluted solutions were used for fortification (determination of recovery rates) and for preparation of the matrix-matched standard solutions used for calibration.

LC-MS/MS chromatographic conditions for Mesosulfuron-methyl determination:

LC-MS/MS system	Autosampler Sciex PAL HTC-xt (ACT Analytics) - Pump LC-20AD XR and Column Oven CTO-20AC (Shimadzu) - Workstation (Analyst 1.6.3)
Column:	Column C18 Hydro RP (50 mm x 2 mm ID; 2.5 µm PD)
Column Temperature:	60°C
Injection Volume:	5 µL for wheat grain and wheat straw 2 µL for wheat whole plant
Mobile phases:	Phase A: Water + 0.1 % glacial acetic acid + 5 mM ammonium acetate Phase B: Methanol + 0.1 % glacial acetic acid + 5 mM ammonium acetate
Gradient:	

Time (min)	Phase A (%)	Phase B (%)	Flow (mL/min)
0	100	0	0.7
0.1	100	0	
4.6	0	100	
6	0	100	
6.1	100	0	

Retention time:	2.9 - 3 min for wheat whole plant and wheat grain 4.1 – 4.2 min for wheat straw.
In these conditions, matrix-matched standard injections of mesosulfuron-methyl showed linear detector response in the ranges from 0.35 to 100 µg/L for wheat whole plant and wheat straw and from 0.9 to 100 µg/L for wheat grain.	

Mass spectrometric conditions

MS System	PE-Sciex API 6500+QTRAP tandem mass Spectrometer
Ion Mode	Positive Multiple Reaction Monitoring (MRM)
Analyte monitored **Mesosulfuron-methyl**	

Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell Time [ms]
504 → 182*	8	33	200 for whole plant and straw, 150 for grain
504 → 139	24	73	

*used as quantifier

Results and discussions

Table A 1: Recovery results from method validation of mesosulfuron-methyl using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 504 → 182 m/z (Primary mass transition)							
Wheat whole plant	0.010	85, 86, 89, 89, 90	88	2	5	88	2
	0.100	86, 89, 88, 85, 89	88	2	5		
Wheat grain	0.010	83, 83, 84, 84, 80	83	2	5	84	2
	0.100	86, 86, 86, 84, 87	86	1	5		
Wheat straw	0.010	91, 89, 88, 95, 92	91	3	5	91	4
	0.100	91, 83, 94, 94, 91	91	5	5		
Ion Mass Transition 504 → 139 m/z (Confirmatory mass transition)							

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Wheat whole plant	0.010	85, 88, 88, 88, 91	88	2	5	88	2
	0.100	85, 89, 89, 84, 87	87	3	5		
Wheat grain	0.010	82, 82, 84, 85, 81	83	2	5	83	2
	0.100	85, 84, 83, 82, 85	84	2	5		
Wheat straw	0.010	90, 91, 84, 96, 90	90	5	5	90	5
	0.100	90, 83, 95, 93, 90	90	5	5		

RSD = Relative Standard Deviation

Table A 2: Characteristics for the analytical method used for validation of mesosulfuron-methyl residues in wheat matrices

	Mesosulfuron-methyl
Specificity	For each matrix, the specificity was checked by the analysis of at least one untreated specimen (two repetitions) and at least one reagent blank. Interferences due to the substrate were less than 30% of the limit of quantification. The solvent blanks showed that no interference due to the reagents was detected. The chromatographic method in LC-MS/MS was highly specific, an additional confirmatory method was not necessary. See representative chromatograms in the report.
Calibration (type, number of data points) **Calibration range**	For each matrix, the linearity range (0.35 to 100 µg/L for whole plant and straw and 0.9 to 100 µg/L for grain) of the method was determined by measuring the detector response (peak area) versus the concentration of a series of at least 5 standard solutions. The linear correlation coefficients were typically higher than 0.99. The analytical calibration extended over a range appropriate to the lowest and highest nominal concentration of the reference item in relevant analytical solutions at least 20%. Wheat whole plant with primary transition: y = 49207.93x-580.68, r = 1.0000. Wheat whole plant with confirmatory transition: y = 9079.00x 92.80, r = 1.0000 Wheat grain with primary transition: y = 131309.22x + 20442.24, r = 0.9992 Wheat grain with confirmatory transition: y = 24381.71x + 1561.01, r = 0.9997 Wheat straw with primary transition: y = 158375,19x + 2042,53, r = 0.9990 Wheat straw with confirmatory transition y = 29691.87x + 444.84, r = 0.9994 Calibration data and the graphs are presented in the report.
Matrix effects	Matrix effects were not investigated. Therefore, all analyses were carried out using matrix-matched standards.
Limit of determination / quantification	The LOQ of the method is the lowest validated level at which a mean recovery within the range 70-110% with a relative standard deviation (RSD) less than or equal to 20% could be obtained. The limit of detection is defined as 30% of the limit of quantification. The LOQ of mesosulfuron-methyl was 0.010 mg/kg corresponding to a LOD of 0.003 mg/kg.

Conclusion

In conclusion, the analytical method has been demonstrated to be a reliable and accurate procedure for the determination of mesosulfuron-methyl in wheat (whole plant, grain, straw) with an LOQ of 0.01 mg/kg for all matrices. The method complies with EU Guideline SANCO/3029/99 rev.4 of 11/07/2000.

A 2.1.1.1.2 Analytical method 2

A 2.1.1.1.2.1 Method validation - Residues of pinoxaden metabolites M4 and M6 in plant matrices

Comments of zRMS:	The analytical method has been fully validated for the determination of pinoxaden metabolites M4 and M6 free and conjugates in wheat (whole plant, grain, straw) with the LOQ of 0.010 mg/kg for M4 in wheat grain and M6 in wheat grain and straw, with an LOQ of 0.02 mg/kg for M4 in whole plant and straw and M6 in whole plant, in

	compliance with SANTE/2020/12830, Rev.1, 24. February 2021. The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than or equal to 20%. It should be noted that this method is similar to the EU agreed method for determination of pinoxaden metabolites. The method is acceptable.

Reference:	KCP 5.1.2/02
Report	Validation of an analytical method for the determination of pinoxaden metabolites M4 and M6 in wheat (whole plant, grain, straw) Barbier G., 2020 Report no. B19G-A4-P-05, ADAMA reference no. 000102680
Guideline(s):	Yes, SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was validated for the determination of pinoxaden metabolites M4 and M6 residues in wheat (whole plant, grain, straw). Residues of pinoxaden metabolites M4 and M6 free and conjugates were extracted together with a mix spiking from homogenised wheat (whole plant, grain, straw) laboratory samples by hydrolysis with 1 N hydrochloric acid under reflux for two hours. Then extracts were purified by solid/liquid purification. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). In order to ensure unambiguous identification, two mass transitions were monitored for pinoxaden metabolites M4 and M6. A LOQ of 0.01 mg/kg was validated for M4 wheat grain and M6 wheat grain and straw and a LOQ of 0.02 mg/kg for M4 whole plant and straw and M6 whole plant.

Test substance	CAS No.	Batch / Lot No.	Purity / content
Pinoxaden metabolite M4	881376-41-6	MES 525/2	91.2%
Pinoxaden metabolite M6	-	MES 585/1	97%

Sample preparation

Extraction: 5 g of ground sample for wheat whole plant and wheat straw and 10 g for wheat grain was weighed into a 250 mL round bottom flask. For wheat grain and wheat straw, 10 mL of water was added. Fortification was performed at this stage. 90 mL of 1M hydrochloric acid for grain and straw and 98.5 mL for whole plant was added to each sample. The sample was heated sample under reflux for two hours.

Liquid/solid purification: After cooling 40 mL of the extract was transferred into a 50 mL centrifuge tube and centrifuged.
5 mL of the centrifuged extract (corresponding to 0.25 g of wheat whole plant and wheat straw and 0.5 g of wheat grain) was transferred onto an OASIS HLB cartridge (without vacuum). The cartridge eluates were discarded. 1 mL of ultra-pure water was added and the cartridge eluates were discarded.
The cartridge was dried and washed with 2 mL of hexane. The eluates were discarded.

For wheat whole plant and wheat grain:
A glass tube was placed under the cartridge and the residues were eluted with 3 mL of dichloromethane/ethyl acetate/formic acid (80/20/0.5, v/v/v) mixture. The cartridge was dried and 500 µL of formic acid 0.5% was added to the eluate and the sample was mixed. The sample was heated to evaporate to the aqueous residue. To receive the final extract the sample was made up to 1 mL with formic acid 0.5%. tract.

For wheat straw:
An evaporation Syncore tube containing 50 µL of ethylene glycol was set up under the cartridge and the residues were eluted with 4 mL of dichloromethane/ethyl acetate/formic acid (80/20/0.5, v/v/v) mixture. After drying the sample was evaporated to the keeper. 950 µL of formic acid 0.5% was added to the eluate and the final extract was mixed and analysed using HPLC-MS/MS.

Standard solutions, Fortification solutions, Matrix-matched calibration solutions:
- Independent standard solutions of M4 and M6 at 10 and 1 mg/L were prepared by dilution of the stock standard solutions in 1 N hydrochloric acid. The standard solutions were stored in a brown flask at a temperature of about + 4°C. These diluted solutions were used for fortification (determination of recovery rates) and for preparation of the matrix-matched standard solutions used for calibration.

LC-MS/MS chromatographic conditions for M4 in wheat (whole plant, grain, straw) and M6 in wheat (whole plant, grain):

LC-MS/MS system	Liquid Chromatograph (LC-MS/MS API 4000) - Autosampler UltiMate 3000 RS (Dionex) - Pump LC UltiMate 3000 RS (Dionex) - Triple quadrupole detector LC/MS/MS API 4000 (Applied Biosystems) - Workstation (Analyst 1.6.2)
Column:	Column C18 Kinetex (100 mm x 4.6 mm ID x 2.6 µm)
Column Temperature:	60°C
Injection Volume:	50 µL, except for M4 in wheat straw 30 µL
Mobile phases:	Phase A: Water + 0.1 % formic acid Phase B: Methanol
Gradient:	

Time (min)	Phase A (%)	Phase B (%)	Flow (mL/min)
0	90	10	0.3
1	90	10	
14	50	50	
14.1	10	90	
15.5	10	90	
15.6	90	10	
21	90	10	

Retention time:	M4: 15.8 -16.3 min M6: 16.7 – 17 min
In these conditions, matrix-matched standard injections of pinoxaden metabolites M4 and M6 showed linear detector response in the ranges from 1.5 to 100 µg/L.	

LC-MS/MS chromatographic conditions for M6 in wheat straw:

LC-MS/MS system	Liquid Chromatograph (LC-MS/MS API 4000) - Autosampler UltiMate 3000 RS (Dionex) - Pump LC UltiMate 3000 RS (Dionex) - Triple quadrupole detector LC/MS/MS API 4000 (Applied Biosystems) - Workstation (Analyst 1.6.2)
Column:	Column C18 Kinetex (100 mm x 4.6 mm ID x 2.6 µm)
Column Temperature:	60°C
Injection Volume:	2 µL
Mobile phases:	Phase A: Water + 2 mM ammonium acetate Phase B: Methanol
Gradient:	

Time (min)	Phase A (%)	Phase B (%)	Flow (mL/min)
0	95	5	0.7
1	0	100	
15.5	0	100	
15.6	95	5	
21	95	5	

Retention time:	M6: about 2.6 min
In these conditions, matrix-matched standard injections of pinoxaden metabolite M6 in wheat straw showed linear detector response in the range from 0.75 to 50 µg/L.	

Mass spectrometric conditions for M4 in wheat (whole plant, grain, straw)

MS System	PE-Sciex API 4000 tandem mass Spectrometer			
Ion Mode	Positive Multiple Reaction Monitoring (MRM)			
Analyte monitored	Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell Time [ms]
M4	333 → 303*	16	33	150
	333 → 101	5	41	

*used as quantifier

Mass spectrometric conditions for M6 in wheat (whole plant, grain, straw)

MS System	PE-Sciex API 4000 tandem mass Spectrometer			
Ion Mode	Negative Multiple Reaction Monitoring (MRM)			
Analyte monitored	Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell Time [ms]
M6	345 → 173*	-9	-44	150
	345 → 158	-9	-56	

*used as quantifier

Results and discussions

Table A 3: Recovery results from method validation of pinoxaden metabolite M4 using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 333 → 303 m/z (Primary mass transition)							
Wheat whole plant	0.020	78, 74, 74, 73, 76	75	3	5	78	5
	0.200	85, 79, 81, 80, 81	81	3	5		
Wheat grain	0.010	78, 75, 94, 95, 90	86	11	5	91	9
	0.100	94, 95, 98, 100, 94	96	3	5		
Wheat straw	0.020	86, 84, 81, 86, 86	85	2	5	88	5
	0.200	89, 84, 91, 97, 91	91	5	5		
Ion Mass Transition 333 → 101 m/z (Confirmatory mass transition)							
Wheat whole plant	0.020	79, 78, 78, 77, 79	78	1	5	80	3
	0.200	85, 79, 82, 82, 81	82	3	5		
Wheat grain	0.010	82, 75, 96, 95, 92	88	11	5	92	9
	0.100	95, 93, 97, 101, 96	97	3	5		
Wheat straw	0.020	85, 87, 84, 86, 87	86	1	5	87	4
	0.200	87, 82, 89, 96, 91	89	5	5		

RSD = Relative Standard Deviation

Table A 4: Recovery results from method validation of pinoxaden metabolite M6 using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 345 → 173 m/z (Primary mass transition)							
Wheat whole plant	0.020	94, 96, 96, 92, 102	96	4	5	98	9
	0.200	109, 86, 87, 110, 107	100	12	5		
Wheat grain	0.010	86, 84, 26[1], 109, 105	96	13	5 (4)	96	12
	0.100	101, 104, 78, 104,98	97	11	5		
Wheat straw	0.010	75, 80, 81, 87, 83	81	5	5	83	7
	0.100	86, 74, 88, 91, 81	84	8	5		
Ion Mass Transition 345 → 158 m/z (Confirmatory mass transition)							
Wheat whole plant	0.020	95, 94, 96, 93, 98	95	2	5	96	9
	0.200	109, 81, 87, 106, 102	97	13	5		
Wheat grain	0.010	83, 82, 25[1], 108, 104	94	14	5 (4)	94	12
	0.100	100, 101, 75, 98, 95	93	12	5		
Wheat straw	0.010	84, 84, 87, 86, 85	85	2	5	85	5
	0.100	86, 76, 86, 92, 82	84	7	5		

RSD = Relative Standard Deviation
1 Sample was eliminated by a Dixon Test with 99 % confidence level. Not used for mean and RSD calculations.

Table A 5: Characteristics for the analytical method used for validation of pinoxaden metabolites M4 and M6 residues in wheat matrices (whole plant, grain, straw)

<table>
<tr><th></th><th>M4</th><th>M6</th></tr>
<tr><td>Specificity</td><td colspan="2">For each reference item and each matrix, the specificity was checked by the analysis of at least one untreated specimen (two repetitions) and at least one reagent blank.
Interferences due to the substrate were less than 30% of the limit of quantification. The solvent blanks showed that no interference due to the reagents were detected. The specificity of the method was demonstrated. See representative chromatograms in the report.

The chromatographic method in LC-MS/MS was highly specific, an additional confirmatory method was not necessary.</td></tr>
<tr><td>Calibration (type, number of data points)
Calibration range</td><td colspan="2">For each reference item and each matrix, the linearity range of the method was determined by measuring the detector response (peak area) versus the concentration of a series of at least 5 standard solutions. The linear correlation coefficients were typically higher than 0.99. The analytical calibration extended over a range appropriate to the lowest and highest nominal concentration of the reference item in relevant analytical solutions ± at least 20%. The lowest calibration solution covered at least 30 % of the LOQ. Calibration data and the graphs are presented in the report.</td></tr>
<tr><td>*primary transition</td><td>Wheat whole plant:
y* = 74974.46x + 15135.97, r = 0.9997
y = 12712.34x + 10700.90, r = 1.0000
Wheat grain:
y* = 55727.25x + 2372.96, r = 0.9996
y = 8002.48x + 602.78, r = 0.9997
Wheat straw:
y* = 17448.27x + 10047.86, r = 0.9983
y = 2872.98x + 1564.61, r = 0.9988</td><td>Wheat whole plant:
y* = 76913.07x + 76998.60, r = 0.9980
y = 53193.40x + 47735.30, r = 0.9983
Wheat grain:
y*= 56901.69x + 2150.64, r = 1.0000
y = 38444.71x + 2880.82, r= 0.9999
Wheat straw
y* = 6323.36x + 278.06, r = 0.9973
y = 4123.93x + 147.50, r = 0.9967</td></tr>
<tr><td>Matrix effects</td><td colspan="2">Guideline SANCO/3029/99 does not request investigation on matrix effects but despite that analyses were carried out using matrix-matched standard, which compensated any matrix effects.</td></tr>
<tr><td>Limit of determination / quantification</td><td colspan="2">The LOQ of the method is the lowest validated level at which a mean recovery within the range 70-110 % with a relative standard deviation (RSD) less than or equal to 20 % could be obtained. The limit of detection is defined as 30 % of the limit of quantification.
<table>
<tr><td></td><td>M4 LOQ/LOD</td><td>M6 LOQ/LOD</td></tr>
<tr><td>Wheat whole plant</td><td>0.020 mg/kg/0.006 mg/kg</td><td>0.020 mg/kg/0.006 mg/kg</td></tr>
<tr><td>Wheat grain</td><td>0.010 mg/kg/0.003 mg/kg</td><td>0.010 mg/kg/0.003 mg/kg</td></tr>
</table></td></tr>
</table>

	M4		M6	
	Wheat straw	0.020 mg/kg/0.006 mg/kg	0.010 mg/kg/0.003 mg/kg	

Conclusion
In conclusion, the analytical method has been demonstrated to be a reliable and accurate procedure for the determination of pinoxaden metabolites M4 and M6 in wheat (whole plant, grain, straw), with an LOQ of 0.01 mg/kg for M4 wheat grain and M6 wheat grain and straw. With an LOQ of 0.02 mg/kg for M4 whole plant and straw and M6 in whole plant. The method complies with EU Guideline SANCO/3029/99 rev.4 of 11/07/2000.

A 2.1.1.1.3 Analytical method 3Method validation - Residues of pinoxaden metabolites M4 and M6 in plant matrices (modified method)

The analytical method for pinoxaden metabolites M4 and M6 was fully validated in 2019 in wheat (grain, straw) in the FREDON Pays de la Loire / GIRPA 2019 study: B19G-A4-P-05, Sponsor reference: 000102680. (See KCP 5.1.2/02).

The analytical method B19G-A4-P-05 was checked again by reduced validation procedure in 2020 in wheat straw within analytical phase of study DMC-20-42727 with poor results. There were issues with the used batch of the cartridges. Therefore, the validation of M4 and M6 in wheat grain was slightly modified and a full validation was performed with a new analytical method.
The summary of this new validation is presented below (see KCP 5.1.2/03).

Comments of zRMS:	In this study the analytical method B19G-A4-P-05 (Barbier G., 2020) has been validated for the determination of pinoxaden metabolites M4 and M6 free and conjugates in wheat grain and straw with the LOQ of 0.010 mg/kg for M4 in wheat grain and M6 in wheat grain and straw, with an LOQ of 0.02 mg/kg for M4 in straw, in compliance with SANTE/2020/12830, Rev.1, 24. February 2021. The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. The method is acceptable.

Reference:	KCP 5.1.2/03
Report	Magnitude of the residue of pinoxaden metabolites, mesosulfuron-methyl, mefenpyr-diethyl and metabolite following one application of ADM.06001.H.2.B in winter wheat in 2 trials (2 HS, one with process), Northern Europe (France and Poland) – 2020 Meric D., 2021 Report no. DMC-20-42727, ADAMA reference no. 000105437
Guideline(s):	Yes, SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The analytical method was validated for the determination of pinoxaden metabolites M4 and M6 residues in wheat (grain and straw). Residues of pinoxaden metabolites M4 and M6 free and conjugates were extracted together with a mix spiking from homogenised wheat (grain and straw) laboratory samples by hydrolysis with 1 N hydrochloric acid under reflux for two hours. Then, wheat extracts were purified by solid/liquid purification. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS or LC-MS). Two mass transitions were monitored for pinoxaden metabolites M4 and M6.

For pinoxaden metabolite M4 the limit of quantification (LOQ) achieved was 0.01 mg/kg for grain and 0.02 mg/kg for straw. For pinoxaden metabolite M6, the LOQ was 0.01 mg/kg for grain and straw. For the sum of M4 and M6 expressed as pinoxaden, the LOQ was 0.024 mg/kg for grain, 0.036 mg/kg for straw. The results are given as M4 or M6 and as their sum expressed as pinoxaden.

Test substance	**CAS No.**	**Batch / Lot No.**	**Purity / content**
Pinoxaden metabolite M4	881376-41-6	MES 525/2	92.2%
Pinoxaden metabolite M6	-	MES 585/1	97%
ADM.06001.H.2.B	-	A8001	Pinoxaden: 61.6 g/L Mesosulfuron-methyl: 12.1 g/l Mefenpyr-diethyl: 36.6 g/L

Sample preparation

Extraction: 5 g of ground sample for wheat straw and 10 g for wheat grain was weighed into a 250 mL round bottom flask. 10 mL of water was added and fortification was performed at this stage. 90 mL of 1M hydrochloric acid was added to each sample and each sample was heated under reflux for two hours.

Liquid/solid purification: After cooling 40 mL of the extract was transferred into a 50 mL centrifuge tube and centrifuged.

- For wheat grain, an aliquot of the supernatant was filtered through a PES 0.2 µm filter into a 2 mL vial. This the final extract.
- For wheat straw, 5 mL of the centrifuged extract (corresponding to 0.25 g of wheat straw) was transferred onto an OASIS HLB cartridge (without vacuum). The cartridge eluates were discarded. 1 mL of ultra-pure water was added and the cartridge eluates were discarded.

The cartridge was dried and washed with 2 mL of hexane. The eluates were discarded.

An evaporation Syncore tube containing 50 µL of ethylene glycol was set up under the cartridge and the residues were eluted with 4 mL of dichloromethane/ethyl acetate/formic acid (80/20/0.5, v/v/v) mixture. After drying the sample was evaporated to the keeper. 950 µL of formic acid 0.5% was added to the eluate and the final extract was mixed and analysed using HPLC-MS/MS.

Standard solutions, Fortification solutions, Matrix-matched calibration solutions:

- Independent standard solutions of M4 and M6 at 10 and 1 mg/L were prepared by dilution of the stock standard solutions in 1 N hydrochloric acid. These diluted solutions were used for fortification (determination of recovery rates) and for preparation of the matrix-matched standard solutions in the range of 0.3 to 30 µg/L, corresponding to 0.003 mg/kg to 0.3 mg/kg used for calibration.

LC-MS/MS chromatographic conditions for M4 in wheat (grain)

<table>
<tr><td>LC-MS/MS system</td><td colspan="4">Liquid Chromatograph (LC-MS/MS API 6500 + QTRAP)
- Autosampler Sciex PAL HTC-xt (ACT Analytics)
- Pump LC-20AD XR (Shimadzu)
- Column Oven CTO-20AC (Shimadzu)
- Triple quadrupole detector 6500+ (Sciex)
- Workstation (Analyst 1.6.3)</td></tr>
<tr><td>Column:</td><td colspan="4">Column C18 Kinetex (100 mm x 4.6 mm ID x 2.6 µm)</td></tr>
<tr><td>Column Temperature:</td><td colspan="4">40°C</td></tr>
<tr><td>Injection Volume:</td><td colspan="4">15 µL</td></tr>
<tr><td>Mobile phases:</td><td colspan="4">Phase A: Water + 0.2 % formic acid
Phase B: acetonitrile</td></tr>
<tr><td>Gradient:</td><td colspan="4"><table><tr><td>Time (min)</td><td>Phase A (%)</td><td>Phase B (%)</td><td>Flow (mL/min)</td></tr><tr><td>0</td><td>95</td><td>5</td><td>0.8</td></tr><tr><td>3</td><td>0</td><td>100</td><td></td></tr><tr><td>5</td><td>0</td><td>100</td><td></td></tr><tr><td>5.1</td><td>95</td><td>5</td><td></td></tr><tr><td>7</td><td>95</td><td>5</td><td></td></tr></table></td></tr>
<tr><td>Retention time:</td><td colspan="4">M4: 2.7 – 2.8 min</td></tr>
<tr><td colspan="5">In these conditions, matrix-matched standard injections of pinoxaden metabolite M4 in wheat grain showed linear detector response in the range from 0.3 to 30 µg/L</td></tr>
</table>

LC-MS/MS chromatographic conditions for M4 in wheat straw:

LC-MS/MS system	Liquid Chromatograph (LC-MS/MS API 4000)

<table>
<tr><td></td><td>- Autosampler UltiMate 3000 RS (Dionex)
- Pump LC UltiMate 3000 RS (Dionex)
- Triple quadrupole detector LC/MS/MS API 4000 (Applied Biosystems)
- Workstation (Analyst 1.6.2)</td></tr>
<tr><td>Column:</td><td>Column C18 Kinetex (100 mm x 4.6 mm ID x 2.6 µm)</td></tr>
<tr><td>Column Temperature:</td><td>60°C</td></tr>
<tr><td>Injection Volume:</td><td>30 µL</td></tr>
<tr><td>Mobile phases:</td><td>Phase A: Water + 0.1% formic acid
Phase B: Methanol</td></tr>
<tr><td>Gradient:</td><td>
<table>
<tr><td>Time (min)</td><td>Phase A (%)</td><td>Phase B (%)</td><td>Flow (mL/min)</td></tr>
<tr><td>0</td><td>90</td><td>10</td><td>0.3</td></tr>
<tr><td>1</td><td>90</td><td>10</td><td></td></tr>
<tr><td>14</td><td>50</td><td>50</td><td></td></tr>
<tr><td>14.1</td><td>10</td><td>90</td><td></td></tr>
<tr><td>15.5</td><td>10</td><td>90</td><td></td></tr>
<tr><td>15.6</td><td>90</td><td>10</td><td></td></tr>
<tr><td>21</td><td>90</td><td>10</td><td></td></tr>
</table>
</td></tr>
<tr><td>Retention time:</td><td>M4: about 15.8 – 16.0 min</td></tr>
<tr><td colspan="2">In these conditions, matrix-matched standard injections of pinoxaden metabolite M4 in wheat straw showed linear detector response in the range from 1.5 to 100 µg/L.</td></tr>
</table>

LC-MS/MS chromatographic conditions for M6 in wheat (grain)

<table>
<tr><td>LC-MS/MS system</td><td>Liquid Chromatograph (LC-MS^n QTrap 7500)
- AD Autosampler Multiplate Exion (Sciex)
- AC Pump Exion (Sciex)
- AC Column Oven Exion (Sciex)
- Triple quadrupole detector 7500 (Sciex)
- Workstation (SciexOS 2.0)</td></tr>
<tr><td>Column:</td><td>Column C18 Kinetex (100 mm x 4.6 mm ID x 2.6 µm)</td></tr>
<tr><td>Column Temperature:</td><td>40°C</td></tr>
<tr><td>Injection Volume:</td><td>3 µL</td></tr>
<tr><td>Mobile phases:</td><td>Phase A: Water + 0.2 % formic acid
Phase B: methanol</td></tr>
<tr><td>Gradient:</td><td>
<table>
<tr><td>Time (min)</td><td>Phase A (%)</td><td>Phase B (%)</td><td>Flow (mL/min)</td></tr>
<tr><td>0</td><td>95</td><td>5</td><td>0.3</td></tr>
<tr><td>14</td><td>20</td><td>80</td><td></td></tr>
<tr><td>14.1</td><td>0</td><td>100</td><td></td></tr>
<tr><td>16</td><td>0</td><td>100</td><td></td></tr>
<tr><td>16.1</td><td>95</td><td>5</td><td></td></tr>
<tr><td>20</td><td>95</td><td>5</td><td></td></tr>
</table>
</td></tr>
<tr><td>Retention time:</td><td>M6: 12.0 – 12.2 min</td></tr>
<tr><td colspan="2">In these conditions, matrix-matched standard injections of pinoxaden metabolite M6 in wheat grain showed linear detector response in the range from 0.3 to 30 µg/L.</td></tr>
</table>

LC-MS/MS chromatographic conditions for M6 in wheat straw:

<table>
<tr><td>LC-MS/MS system</td><td>Liquid Chromatograph (LC-MS/MS API 4000)
- Autosampler UltiMate 3000 RS (Dionex)
- Pump LC UltiMate 3000 RS (Dionex)
- Triple quadrupole detector LC/MS/MS API 4000 (Applied Biosystems)
- Workstation (Analyst 1.6.2)</td></tr>
<tr><td>Column:</td><td>Column C18 Kinetex (100 mm x 4.6 mm ID x 2.6 µm)</td></tr>
<tr><td>Column Temperature:</td><td>60°C</td></tr>
<tr><td>Injection Volume:</td><td>2 µL</td></tr>
<tr><td>Mobile phases:</td><td>Phase A: Water + ammonium acetate
Phase B: Methanol</td></tr>
<tr><td>Gradient:</td><td>
<table>
<tr><td>Time (min)</td><td>Phase A (%)</td><td>Phase B (%)</td><td>Flow (mL/min)</td></tr>
<tr><td>0</td><td>95</td><td>5</td><td>0.7</td></tr>
<tr><td>3</td><td>0</td><td>100</td><td></td></tr>
<tr><td>5</td><td>0</td><td>100</td><td></td></tr>
<tr><td>5.1</td><td>95</td><td>5</td><td></td></tr>
<tr><td>7</td><td>95</td><td>5</td><td></td></tr>
</table>
</td></tr>
<tr><td>Retention time:</td><td>M4: about 2.3 – 2.6 min</td></tr>
<tr><td colspan="2">In these conditions, matrix-matched standard injections of pinoxaden metabolite M6 in wheat straw showed linear detector response in the range from 0.75 to 50 µg/L.</td></tr>
</table>

Mass spectrometric conditions for M4 in wheat grain

MS System	PE-Sciex API 6500 + QTRAP tandem mass Spectrometer			
Ion Mode	Positive Multiple Reaction Monitoring (MRM)			
Analyte monitored	Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell Time [ms]
M4	333 → 303*	16	33	250
	333 → 101	5	41	250

*used as quantifier

Mass spectrometric conditions for M6 in wheat grain

MS System	PE-Sciex 7500 QTRAP tandem mass Spectrometer		
Ion Mode	Negative MS^3		
Analyte monitored	Mass transition [m/z]	Collision Energy [V]	Auxiliary frequency 2 [V]
M6	345 → 173* for straw 345 →217→ 173* for grain	-37	0.24
	345 → 158 for straw 345 →173→ 158 for grain	-46	0.34

*used as quantifier

Mass spectrometric conditions for M4 in wheat straw

MS System	PE-Sciex API 4000 tandem mass Spectrometer			
Ion Mode	Positive Multiple Reaction Monitoring (MRM)			
Analyte monitored	Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell Time [ms]
M4	333 → 303*	16	33	150
	333 → 101	5	41	

*used as quantifier

Mass spectrometric conditions for M6 in wheat straw

MS System	PE-Sciex API 4000 tandem mass Spectrometer			
Ion Mode	Negative Multiple reaction Monitoring (MRM)			
Analyte monitored	Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell tome (ms)
M6	345 → 173*	-9	-44	150
	345 → 158	-9	-45	150

*used as quantifier

Results and discussions

The accuracy of the method fulfils the requirements for residue analytical methods which demand that the mean recovery per fortification level is in the range 70 - 110%.

Table A 6: Recovery results from method validation of pinoxaden metabolite M4 using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 333 → 303 m/z (Primary mass transition)							
Wheat grain	0.010	88, 88, 88, 87, 87	88	1	5	88	1
	0.100	86, 90, 90, 89, 89	89	2	5		
Wheat straw	0.020	82, 86, 83	84	2	3	82	3
	0.200	82, 82, 77	81	3	3		

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 333 → 101 m/z (Confirmatory mass transition)							
Wheat grain	0.010	96, 90, 96, 88, 95	93	3	5	90	5
	0.100	84, 88, 87, 86, 86	86	2	5		
Wheat straw	0.020	80, 86, 84	83	3	3	82	3
	0.200	81, 81, 77	80	3	3		

RSD = Relative Standard Deviation

Table A 7: Recovery results from method validation of pinoxaden metabolite M6 using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 345 → 217→ 173 m/z (Primary mass transition)							
Wheat grain	0.010	72, 91, 69, 73, 77	77	10	5	77	7
	0.100	77, 76, 79, 83, 76	78	3	5		
Ion Mass Transition 345 → 173 m/z (Primary mass transition)							
Wheat straw	0.010	77, 70, 87	78	9	3	76	7
	0.100	77, 74, 74	75	2	3		
Ion Mass Transition 345 → 173 → 158 m/z (Confirmatory mass transition)							
Wheat grain	0.010	72, 72, 83, 82, 57	73	13	5	77	10
	0.100	79, 83, 81, 78, 83	81	2	5		
Ion Mass Transition 345 → 158 m/z (Confirmatory mass transition)							
Wheat straw	0.010	79, 70, 85	78	8	3	75	7
	0.100	74, 73, 71	75	2	3		

RSD = Relative Standard Deviation

Table A 8: Characteristics for the analytical method used for validation of pinoxaden metabolites M4 and M6 residues in wheat matrices (grain and straw)

	M4	M6
Specificity	For each reference item and each matrix, the specificity was checked by the analysis of at least one untreated specimen (two repetitions) and at least one reagent blank. Interferences due to the substrate were less than 30% of the limit of quantification. The solvent blanks showed that no interference due to the reagents were detected. The specificity of the method was demonstrated. See representative chromatograms in the report. The chromatographic method in LC-MS/MS or LC-MS^3 was highly specific, an additional confirmatory method was not necessary.	
Calibration (type, number of data points) Calibration	For each reference item and each matrix, the linearity range of the method was determined by measuring the detector response (peak area) versus the concentration of a series of at least 5 standard solutions. The linear correlation coefficients were typically higher than 0.99. The concentration range covered from 30 % of the LOQ to 20 % above the highest level. Calibration data and the graphs are presented in the report.	

<table>
<tr><td></td><td>M4</td><td>M6</td></tr>
<tr><td>range

*primary transition</td><td>Wheat grain:
y* = 261500.8098 x + 16192.9345, r = 1.0000
y = 30344.4136 x + 1594.9917, r = 1.0000 Wheat straw:
y* = 31012.7865 x – 5187.1805, r = 0.9998
y = 5831.9559 x – 301.9556, r = 0.9998</td><td>Wheat grain:
y*= 14542050.1956 x + 1262217.6903, r = 0.9991
y = 16888215.4885 x + 1702994.1054, r = 0.9987
Wheat straw
y* = 18853.6014 x – 1168.6489, r = 0.9997
y = 12427.7058 x – 1379.7712, r = 0.9998</td></tr>
<tr><td>Matrix effects</td><td colspan="2">Guideline SANCO/3029/99 does not request investigation on matrix effects but despite that analyses were carried out using matrix-matched standard, which compensated any matrix effects.</td></tr>
<tr><td>Limit of determination / quantification</td><td colspan="2">The LOQ of the method is the lowest validated level at which a mean recovery within the range 70-110 % with a relative standard deviation (RSD) less than or equal to 20 % could be obtained. The limit of detection is defined as 30 % of the limit of quantification.
<table>
<tr><td></td><td>M4 LOQ/LOD</td><td>M6 LOQ/LOD</td><td>Sum of M4 + M6 expressed as pinoxaden LOQ/LOD</td></tr>
<tr><td>Wheat grain</td><td>0.010 mg/kg/0.003 mg/kg</td><td>0.010 mg/kg/0.003 mg/kg</td><td>0.024 mg/kg / 0.007 mg/kg</td></tr>
<tr><td>Wheat straw</td><td>0.020 mg/kg/0.006 mg/kg</td><td>0.010 mg/kg/0.003 mg/kg</td><td>0.036 mg/kg / 0.011 mg/kg</td></tr>
</table></td></tr>
<tr><td>Stability in final sample extracts</td><td>M4 in wheat grain, the final sample extracts were analysed within 24 hours after initial extraction. Therefore, no stability study was performed.</td><td>It was shown, that under refrigerated conditions, final sample extracts were considered stable:
- for M6 in wheat grain for at least 15 days
- for M4 in wheat straw for at least 5 days
- for M6 in wheat straw for at least 7 days
Thus, covering the storage durations observed within this analytical phase (<15 days for M6 in wheat grain, < 6 days for M4 and < 8 days for M6 in wheat straw).</td></tr>
</table>

Conclusion
In conclusion, the analytical method has been demonstrated to be a reliable and accurate procedure for the determination of pinoxaden metabolites M4 in wheat grain and M6 in wheat grain and straw with an LOQ of 0.01 mg/kg and for M4 wheat straw with an LOQ of 0.02 mg/kg. The method complies with EU Guideline SANCO/3029/99 rev.4 of 11/07/2000.

A 2.1.1.1.4 Analytical method 4

A 2.1.1.1.4.1 Method validation - Residues of mefenpyr-diethyl in plant matrices

Comments of zRMS:	The analytical method (QuEChERS-method) has been validated for selectivity, linearity, accuracy and precision for the determination of mefenpyr-diethyl and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid in wheat whole plant, grain and straw with the LOQ of 0.010 mg/kg for both compounds and for each matrix, in compliance with SANCO/3029/99 rev.4 . The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. Remark: According to the SANTE/2020/12830 rev.1 the extraction efficiency should be demonstrated for this method. It should be noted that this method uses a different extraction than in method used in the metabolism study and does not include a hydrolysis step. However, since the study is based on a method reviewed at EU level (01300-M001), it is considered acceptable. The method is acceptable.

Reference: KCP 5.1.2/04

Report: Validation of an analytical method for the determination of mefenpyr-diethyl in wheat (whole plant, grain, straw)

	Lefresne S., 2019 Study code: B19S-A4-M-01, ADAMA reference no. 000102679
Guideline(s):	Yes, SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method principle of the QuEChERS-method was used for residue determination of mefenpyr-diethyl in wheat (whole plant, grain, straw). The sample work up includes acetonitrile extraction/partitioning and clean-up using dispersive SPE followed by LC-MS/MS analysis.

Residues of mefenpyr-diethyl and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid expressed as mefenpyr-diethyl were extracted from homogenised specimens by maceration with acetonitrile acidified with 0.2M sulphuric acid and water. Then, extracts were purified by dispersive solid phase extraction. The quantification was performed by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

Test substance	CAS No.	Batch / Lot No.	Purity / content
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.5 ± 1.4 %
Metabolite 1-(2,4-dichloro-phenyl)-5-methyl-pyrazole-3-carboxylic acid	26067-48-9	251-3463	94.7%,

Sample preparation

2 g of ground laboratory sample was weighed into a 50 mL centrifuge tube. Fortification was performed at this stage. 10 mL of ultra-pure water and 10 mL of acetonitrile acidified with 0.2M H_2SO_4 was added and the sample was shaken. One sachet of $MgSO_4$/NaCl/salts tampon was added to the centrifuge tube.

A 5 mL aliquot of the supernatant (corresponding to 1 g of matrix) was transferred into an evaporation tube. 50 µL of ethylene glycol was added as keeper. Evaporate just at the keeper and dissolve the residue into 1 mL of acetonitrile. The quantification was performed by HPLC.MS/MS.

Standard solutions, Fortification solutions, Matrix-matched calibration solutions:

Standard solutions, Fortification solutions, Matrix-matched calibration solutions:

- Mixture standard solutions at 10 and 1 mg/L of each reference item expressed as mefenpyr-diethyl (see note) were prepared by dilution of the stock standard solutions in acetonitrile. The standard solutions were stored in a brown flask at a temperature of about - 18°C.

These diluted solutions were used for fortification (determination of recovery rates) and for preparation of the matrix-matched standard solutions used for calibration.

Note: taking into account the molar masses of each compound, for example a mass m of 3- metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid corresponds to a mass (373.2/271.1) m = 1.377 m of mefenpyr-diethyl equivalent.

LC-MS/MS chromatographic conditions for Mefenpyr-diethyl and Metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid determination

LC system	Liquid Chromatograph (LC-MS/MS 6500+) - Autosampler Sciex PAL HTC-xt (ACT Analytics) - Pump LC-20AD XR (Shimadzu) - Column Oven CTO-20AC (Shimadzu) - Triple quadrupole detector 6500+ (Sciex) - Workstation (Analyst 1.6.3)
Column:	Column C18 Hydro RP (100 mm x 3 mm ID x 2.5 µm)
Column Temperature:	40°C

Injection Volume:	10 µL
Mobile phases:	Phase A: Ultra-pure water + 0.1 % glacial acetic acid + 5 mM ammonium acetate Phase B: Methanol + 0.1 % glacial acetic acid + 5 mM ammonium acetate
Gradient:	Time (min) / Phase A (%) / Phase B (%) / Flow (mL/min) 0.0 / 100 / 0 / 0.7 3.0 / 0 / 100 5.0 / 0 / 100 5.5 / 100 / 0 7.0 / 100 / 0
Retention time:	Mefenpyr-diethyl about 4.1 minutes and Metabolite 1-(2,4-dichlorophenyl)5-methy-l-pyrazole-3-carboxylic acid About 3.5 minutes
In these conditions, matrix-matched standard injections of mefenpyr-diethyl and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid expressed as mefenpyr-diethyl showed linear detector response in the range of 3 to 200 µg/L	

Mass spectrometric conditions

MS System	PE-Sciex API 4000 tandem mass Spectrometer			
Ion Mode	Positive Multiple Reaction Monitoring (MRM)			
Analytes monitored **Mefenpyr-diethyl-1 *** **Mefenpyr-diethyl-2** **Metabolite of mefenpyr-diethyl *** **Metabolite of mefenpyr-diethyl**	Mass transition [m/z]	Collision cell eXit Potential [V]	Collision Energy [V]	Dwell Time [ms]
	373 → 160*	14	45	100
	373 → 133	12	71	
	271 → 189*	16	51	
	271 → 253	14	21	

*used as quantifier

Results and discussions

Table A 9: Recovery results from method validation of mefenpyr-diethyl using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 373 → 160 m/z (Primary mass transition)							
Wheat whole plant	0.010	95, 89, 97, 92, 99	94	4	5	96	4
	0.100	101, 94, 100, 92, 97	97	4	5		
Wheat grain	0.010	91, 74, 85, 84, 81	83	7	5	85	6
	0.100	90, 87, 89, 81, 84	86	4	5		
Wheat straw	0.010	107, 87, 73, 95, 94	91	14	5	90	10
	0.100	91, 84, 95, 88, 82	88	6	5		
Ion Mass Transition 373 → 133 m/z (Confirmatory mass transition)							
Wheat whole plant	0.010	95, 92, 99, 95, 99	96	3	5	96	4
	0.100	102, 96, 98, 91, 99	97	4	5		
Wheat grain	0.010	92, 71, 87, 86, 81	83	10	5	85	7
	0.100	90, 86, 87, 81, 84	86	4	5		
Wheat straw	0.010	97, 96, 73, 83, 109	92	15	5	90	11
	0.100	90, 83, 95, 88, 82	88	6	5		

RSD = Relative Standard Deviation

Table A 10: Recovery results from method validation of metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3carboxylic acid expressed as mefenpyr-diethyl using the analytical method

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Control samples		<30 % LOQ	-	-	2		
Reagent blank		<30 % LOQ	-	-	1		
Ion Mass Transition 271 → 189 m/z (Primary mass transition)							
Wheat whole plant	0.010	92, 90, 102, 86, 86	91	7	5	95	6
	0.100	102, 93, 97, 99, 98	98	3	5		
Wheat grain	0.010	76, 75, 85, 86, 77	80	6	5	83	6
	0.100	83, 90, 86, 87, 84	86	3	5		
Wheat straw	0.010	78, 82, 75, 86, 98	84	11	5	88	10
	0.100	96, 86, 102, 92, 87	93	7	5		
Ion Mass Transition 271 → 253 m/z (Confirmatory mass transition)							
Wheat whole plant	0.010	95, 93, 98, 88, 90	93	4	5	96	4
	0.100	100, 96, 100, 98, 98	99	2	5		
Wheat grain	0.010	82, 74, 85, 82, 80	81	5	5	74	6
	0.100	84, 91, 89, 90, 86	88	3	5		
Wheat straw	0.010	95, 98, 92, 92, 96	95	3	5	92	5
	0.100	93, 90, 94, 89, 82	90	5	5		

RSD = Relative Standard Deviation

Table A 11: Characteristics for the analytical method used for validation of mefenpyr-diethyl and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3carboxylic acid expressed as mefenpyr-diethyl residues in wheat matrices (whole plant, grain, straw)

	Mefenpyr-diethyl	1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3carboxylic acid expressed as mefenpyr-diethyl
Specificity	For each reference item and each matrix, the specificity was checked by the analysis of at least one untreated specimen (two repetitions) and at least one reagent blank. Interferences due to the substrate were less than 30% of the limit of quantification. The solvent blanks showed that no interference due to the reagents were detected. The specificity of the method was demonstrated. Representative chromatograms are presented in the report. The chromatographic method in LC-MS/MS was highly specific, an additional confirmatory method was not necessary.	
Calibration (type, number of data points) **Calibration range** ***primary transition**	For each reference item and each matrix, the linearity range (3 to 200 µg/L) of the method was determined by measuring the detector response (peak area) versus the concentration of a series of at least 5 standard solutions. The linear correlation coefficients were typically higher than 0.99. The analytical calibration extended over a range appropriate to the lowest and highest nominal concentration of the reference item in relevant analytical solutions ± at least 20%. The lowest calibration solution covered at least 30 % of the LOQ. Calibration data and the graphs are presented in the report.	
	Wheat whole plant: y* = 6561.70x + 60.75, r = 0.9995 y = 4717.34x – 1200.60, r = 0.9997 Wheat grain: y* = 14175.84x + 404.57, r = 0.9993 y = 10263.16x + 2223.05, r = 0.9995 Wheat straw: y* = 1808.19x + 739.04, r = 0.9993 y = 1310.70x – 319.48, r = 0.9991	Wheat whole plant: y* = 832.76x – 1483.15, r = 0.9991 y = 9939.88x – 16027.13, r = 0.9982 Wheat grain: y*= 3264.32x – 4419.64, r = 0.9983 y = 35123.77x – 39664.76, r= 0.9994 Wheat straw y* = 186.99x – 281.72, r = 0.9996 y = 2352.62x – 3051.67, r = 0.9967
Matrix effects	For each reference item and each matrix, matrix effects were not investigated. Therefore, all	

	Mefenpyr-diethyl	**1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3carboxylic acid expressed as mefenpyr-diethyl**
	analyses were carried out using matrix-matched standards.	
Limit of determination / quantification	The LOQ of the method is the lowest validated level at which a mean recovery within the range 70-110 % with a relative standard deviation (RSD) less than or equal to 20 % could be obtained. The limit of detection is defined as 30 % of the limit of quantification. The LOQ of mefenpyr-diethyl and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid expressed as mefenpyr-diethyl was 0.010 mg/kg for each reference item corresponding to a LOD of 0.003 mg/kg.	

Conclusion
In conclusion, the analytical method has been demonstrated to be a reliable and accurate procedure for the determination of mefenpyr-diethyl and metabolite 1-(2,4-dichlorophenyl)-5-methyl-pyrazole-3-carboxylic acid expressed as mefenpyr-diethyl residues in wheat (whole plant, grain and straw). A LOQ of 0.01 mg/kg was validated for each analyte and each matrix. The method complies with EU Guideline SANCO/3029/99 rev.4 of 11/07/2000.

Lefresne S., 2019

A 2.1.1.1.5 Analytical method 5

A 2.1.1.1.5.1 Method validation - Acute toxicity to aquatic invertebrates (*Daphnia magna)*

Comments of zRMS:	The analytical method has been validated for determination of mesosulfuron-methyl, pinoxaden, pinoxaden M2 and mefenpyr-diethyl in Elendt M4 medium treated with ADM.06001.H.2.B in compliance with SANCO/3029/99 rev.4 and SANTE/2020/12830 rev. 1. The limit of quantification (LOQ) for the test item concentration was determined to be the fortification level of nominal 0.2 mg test item/L (1.9 µg mesosulfuron-methyl/L, 10.1 µg pinoxaden/L and 6.1 µg mefenpyr-diethyl/L). The limit of quantification for the M2 (metabolite of pinoxaden) concentration was determined to be the fortification level of nominal 2 µg M2 (metabolite of pinoxaden)/L (1.6 µg M2/L). The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. The method is acceptable.

Reference: KCP 5.1.2/05 (also filed under KCP 10.2.1/01)

Report: ADM.06001.H.2.B: Acute Toxicity to *Daphnia magna* in a Semi-Static 48-hour Immobilisation Test
Seidel U. and Mollandin G., 2021a
Report no: 140711220, ADAMA reference no: 000105363

Guideline(s): SANCO/3029/99 rev. 4

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods
The analytical method was validated for determination of mesosulfuron-methyl, pinoxaden, pinoxaden M2 and mefenpyr-diethyl in Elendt M4 medium coming from ecotoxicological tests with *Daphnia magna* with the test item ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	232-3283, purity: 99.4% G141997	99.4
Pinoxaden (PNX-400)	243973-20-8	257-3521	99.3%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	98.7%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.82%

Standard Solutions used for Quantification

Stock Solution: The analytical reference item was used to prepare separate stock solutions dissolved in acetonitrile of approximately 1 g analytical reference item/L. Therefore, 20.02 mg of the mesosulfuron-methyl analytical reference item were dissolved in 20 mL acetonitrile, 20.02 mg of the pinoxaden analytical reference item were dissolved in 20 mL acetonitrile containing 0.3% formic acid, 10.05 mg of the M2 (metabolite of pinoxaden) analytical reference item were dissolved in 10 mL acetonitrile and 20.10 mg of the and mefenpyr-diethyl analytical reference item were dissolved in 20 mL acetonitrile.

Standard Solutions:

Appropriate amounts of the different stock solutions were diluted with acetonitrile to obtain a mixed standard solution of 10 mg mesosulfuron-methyl/L, 15 mg pinoxaden/L, 10 mg M2 (metabolite of pinoxaden)/L and 10 mg mefenpyr-diethyl/L. Appropriate amounts of this mixed standard solution were diluted further with test water at pH 4 / acetonitrile (4/1, v/v) to obtain standard solutions in the range of 0.5 to 30 µg mesosulfuron-methyl/L, 0.75 to 45 µg pinoxaden/L, 0.5 to 30 µg M2 (metabolite of pinoxaden)/L and 0.5 to 30 µg mefenpyr-diethyl/L.

Fortified Samples:

50 mg of the test item and 0.5 mL of 1 g M2 (metabolite of pinoxaden)/L stock solution were dissolved in 100 mL acetonitrile to obtain a stock solution of approximately 0.5 g test item/L spiked with 5 mg M2 (metabolite of pinoxaden)/L. Two independent stock solutions were prepared. Appropriate amounts of these stock solutions were diluted with test water at pH 4 / acetonitrile (4/1, v/v) to obtain fortified samples at a level of 0.2 and 120 mg test item/L and corresponding the resulting level of 2.0 and 1200 µg M2 (metabolite of pinoxaden)/L.

Stability Samples:

Pinoxaden, M2 (metabolite of pinoxaden) and mefenpyr-diethyl were analysed within 21 days of test start. Mesosulfuron-methyl were not analysed within 30 days of test start. Therefore, verification of the storage stability was necessary only for mesosulfuron-methyl.

LC-MS/MS chromatographic conditions for Mesosulfuron-methyl, Mefenpyr-diethyl, Pinoxaden and Pinoxaden M2 determination

LC-MS/MS system	Agilent Series 1290 pump and autosampler
Column	Phenomenex Synergi 4 µm Fusion-RP 80A (50x2 mm)
Column Temperature	40°C
Injection Volume	5 µL
Mobile phases	Eluent A: HPLC-water + 0.1 % HCOOH Eluent B: acetonitrile + 0.1 % HCOOH
Gradient	

Time (min)	%Eluent A	% Eluent B	Flow (mL/min)
0	95	5	0.65
2	95	5	0.65
2.5	50	50	0.65
4.5	5	95	0.65
5.2	5	95	0.65
5.3	95	5	0.65

	7	95	5	0.65
Run time	7 minutes			
MS System	Mass spectrometer API 5500			
Ion source	Electrospray ionization (ESI) positive			
Curtain gas (CUR): **Nebulizer gas (GS1):**	30 psi 70 psi			
Voltage (IS):	5500 V			
Heater Temperature (TEM)	350 °C for mesosulfuron-methyl and mefenpyr-diethyl 475 °C for pinoxaden and M2 (metabolite of pinoxaden)			
Turbo gas (GS2): **Collision gas (CAD):**	60 psi 8			
Dwell time:	150 ms for mesosulfuron-methyl and mefenpyr-diethyl 100 ms for pinoxaden and M2 (metabolite of pinoxaden)			

MRM mass Transition

Analyte	**Precursor ion [m/z]**	**Product ion [m/z]**	**Declustering Potential (DP) [V]**	**Entrance Potential (EP) [V]**	**Collision Energy (V)**	**Collision Exit Potential (CXP) [V]**
Mesosulfuron-methyl (Q1)	504.1	182.1	75	12	30	10
Mesosulfuron-methyl (Q2)	504.1	83	75	12	100	40
Mefenpyr-diethyl (Q1)	389.9	327.0	75	2	20	5
Mefenpyr-diethyl (Q2)	389.9	160.0	75	2	47	5
Pinoxaden (Q1)	401.5	317.2	72	10	33	22
Pinoxaden (Q2)	401.5	56.9	72	10	49	8
M2 metabolite of pinoxaden (Q1)	317.8	171.2	101	10	33	10
M2 metabolite of pinoxaden(Q2)	317.8	131.1	101	10	57	8

Quantifier (Q1) transitions were used for quantification
Qualifier (Q2) transitions were used for confirmation. Qualifier were monitored but not evaluated.

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Table A 12: Recovery results from method validation of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden, and pinoxaden M2 in Elendt M4 medium

Matrix	**Analyte**	**Fortification level (mg a.s./L)***	**Recovery (%)****	**n**	**Recovery Range (%)**	**Mean Recovery (%)**	**RSD (%)**	**Comments**
Elendt M4 medium	Mesosulfuron-methyl	0.2	111, 108, 111, 106, 104	5	104-111	108	3	-
		120	108, 107, 112, 107, 107	5	107-112	108	2	-
			Overall	10	104-112	108	2	-
	Mefenpyr-diethyl	0.2	107, 96, 95, 99, 97	5	95-107	99	5	-
		120	106, 102, 100, 103, 98	5	98-106	102	3	-
			Overall	10	98-107	100	4	-
	Pinoxaden	0.2	109, 105, 107, 105, 100	5	100-109	105	3	-
		120	102, 99, 100, 101, 97	5	97-102	100	2	-
			Overall	10	97-109	102	4	-
	Pinoxaden M2	2.00 µg/L	98, 103, 101, 101, 107	5	98-107	102	4	-
		1200 µg/L	111, 102, 101, 108, 98	5	98-111	104	5	-
			Overall	10	98-111	103	4	-

*Limit of quantification, defined by the lowest validated fortification level.
**Residues in control and reagent blank samples were less than 30% of the LOQ
RSD: Relative Standard Deviation

Table A 13: Characteristics for the analytical method used for validation of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and pinoxaden M2 in Elendt M4 medium

	Mesosulfuron-methyl, Pinoxaden, Pinoxaden M2 and Mefenpyr-diethyl
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Linearity of Response: Correlation of peak area of different standard solutions (nine levels) with their corresponding concentrations, using a linear regression Calibration range: Mesosulfuron-methyl: 0.5 – 30 µg analytical reference item/L Pinoxaden: 0.75 – 45 µg analytical reference item/L Pinoxaden M2: 0.5 – 30 µg analytical reference item/L Mefenpyr-diethyl: 0.5 – 30 µg analytical reference item/L Calibration Curve (linear regression): Mesosulfuron-methyl: y=77840*x+2193, r = 0.9999 Pinoxaden: y=222747*x+228782, r = 0.9990 Pinoxaden M2: y=3401*x+57, r=0.9999 Mefenpyr-diethyl: y=3158*x+1256, r = 0.9994 For all analytes, calibration data and graphs are presented in the report.
Matrix effects	Not relevant, not tested
Limit of determination (LOD)	Mesosulfuron-methyl: 0.003 µg mesosulfuron-methyl/L Pinoxaden: 0.004 µg pinoxaden/L M2 (metabolite of pinoxaden): 0.281 µg M2/L Mefenpyr-diethyl: 0.156 µg mefenpyr-diethyl/L
Limit of Quantification (LOQ)	Fortification level of nominal 0.2 mg test item/L, corresponding to the following concentrations of the active substances and the safener analysed after dilution by a factor of 1.25: 1.9 µg mesosulfuron-methyl/L, 10.1 µg pinoxaden/L and 6.1 µg mefenpyr-diethyl/L. And for the metabolite of pinoxaden the fortification level of nominal 2 µg M2/L, corresponding to the following concentration of M2 analysed after dilution by a factor of 1.25: 1.6 µg M2/L.

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and pinoxaden M2 in Elendt M4 medium treated with ADM.06001.H.2.B.

A 2.1.1.1.6 Analytical method 6

A 2.1.1.1.6.1 Method validation - Effects on aquatic algae

Comments of zRMS:	The analytical method has been validated for determination of mesosulfuron-methyl, pinoxaden, pinoxaden M2 and mefenpyr-diethyl in OECD medium in compliance with SANCO/3029/99 rev.4 and SANTE/2020/12830 rev. 1. The limit of quantification (LOQ) for the test item concentration was determined to be the fortification level of nominal 0.05 mg test item/L. The limit of quantification for the M2 (metabolite of pinoxaden) concentration was determined to be the fortification level of nominal 1.5 µg M2 (metabolite of pinoxaden)/L. The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. The method is acceptable.

Reference: KCP 5.1.2/06 (also filed under KCP 10.2.1/02)

Report: ADM.06001.H.2.B: Toxicity to Raphidocelis subcapitata (=*Pseudokirchneriella subcapitata*) in an Algal Growth Inhibition Test
Seidel U. and Mollandin G., 2021b
Report no: 140711210, ADAMA reference no: 000105364

Guideline(s): SANCO/3029/99 rev. 4

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method was validated for determination of mesosulfuron-methyl, pinoxaden, pinoxaden M2 and mefenpyr-diethyl in OECD Medium coming from ecotoxicological tests with *Raphidocelis subcapitata (=Pseudokirchneriella subcapitata)* with the test item ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	232-3283	99.6%
Pinoxaden (PNX-400)	243973-20-8	257-3521	99.3%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	98.7%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.82%

Standard Solutions used for Quantification

Stock Solution: The analytical reference item was used to prepare separate stock solutions dissolved in acetonitrile of approximately 1 g analytical reference item/L. Therefore, 20.03 mg of the mesosulfuron-methyl analytical reference item were dissolved in 20 mL acetonitrile, 20.02 mg of the pinoxaden analytical reference item were dissolved in 20 mL acetonitrile containing 0.3% formic acid, 50.29 mg of the M2 (metabolite of pinoxaden) analytical reference item were dissolved in 50 mL acetonitrile and 20.10 mg of the and mefenpyr-diethyl analytical reference item were dissolved in 20 mL acetonitrile.

Standard Solutions:

Appropriate amounts of the stock solutions were diluted with acetonitrile to obtain a mixed analytical standard solution of 10 mg mesosulfuron-methyl/L, 15 mg pinoxaden/L, 10 mg M2 (metabolite of pinoxaden)/L and 10 mg mefenpyr-diethyl/L. Appropriate amounts of this mixed standard solution were diluted further with test water at pH 4 / acetonitrile (4/1, v/v) to obtain standard solutions in the range from 0.125 to 60 µg mesosulfuron-methyl/L, 0.1875 to 90 µg pinoxaden/L, 0.125 to 60 µg M2 (metabolite of pinoxaden)/L and 0.125 to 60 µg mefenpyr-diethyl/L.

Fortified Samples:
50 mg of the test item and 1.5 mL of 1 g M2 (metabolite of pinoxaden)/L stock solution were dissolved in 100 mL acetonitrile to obtain a stock solution of approximately 0.5 g test item/L spiked with 15 mg M2 (metabolite of pinoxaden)/L. Two independent stock solutions were prepared. Appropriate amounts of these stock solutions were diluted with test water at pH 4 / acetonitrile (4/1, v/v) to obtain fortified samples at a level of 0.05, 0.6 and 110 mg test item/L and corresponding to the resulting level of 0.0015, 0.018 and 3.3 mg M2 (metabolite of pinoxaden)/L.

LC-MS/MS chromatographic conditions for Mesosulfuron-methyl, Mefenpyr-diethyl, Pinoxaden and Pinoxaden M2 determination

LC-MS/MS system	Agilent Series 1290 pump and autosampler
Column	Phenomenex Synergi 4 µm Fusion-RP 80A (50x2 mm)
Column Temperature	40°C
Injection Volume	20 µL for mesosulfuron-methyl, mefenpyr-diethyl and M2 (metabolite of pinoxaden) 2 µL for pinoxaden:
Mobile phases	Eluent A: HPLC-water + 0.1 % HCOOH Eluent B: acetonitrile + 0.1 % HCOOH
Gradient	Time (min) / %Eluent A / % Eluent B / Flow (mL/min) 0 / 95 / 5 / 0.65 2 / 95 / 5 / 0.65 2.5 / 50 / 50 / 0.65 4.5 / 5 / 95 / 0.65 5.2 / 5 / 95 / 0.65 5.3 / 95 / 5 / 0.65 7 / 95 / 5 / 0.65
Run time	7 minutes
MS System	Mass spectrometer API 5500
Ion source	Electrospray ionization (ESI) positive
Curtain gas (CUR): **Nebulizer gas (GS1):**	30 psi 70 psi
Voltage (IS):	5500 V
Heater Temperature (TEM)	350 °C for mesosulfuron-methyl, mefenpyr-diethyl and pinoxaden M2 (metabolite of pinoxaden) 475 °C for pinoxaden
Turbo gas (GS2): **Collision gas (CAD):**	60 psi 8
Dwell time:	150 ms for mesosulfuron-methyl, mefenpyr-diethyl and pinoxaden M2 (metabolite of pinoxaden) 100 ms for pinoxaden
MRM mass Transition	

Analyte	Precursor ion [m/z]	Product ion [m/z]	Declustering Potential (DP) [V]	Entrance Potential (EP) [V]	Collision Energy (V)	Collision Exit Potential (CXP) [V]
Mesosulfuron-methyl (Q1)	504.1	182.1	75	12	30	10
Mesosulfuron-methyl (Q2)	504.1	83	75	12	100	40
Mefenpyr-diethyl (Q1)	389.9	327.0	75	2	20	5
Mefenpyr-diethyl (Q2)	389.9	160.0	75	2	47	5
Pinoxaden (Q1)	401.5	317.2	72	10	33	22
Pinoxaden (Q2)	401.5	56.9	72	10	49	8
M2 metabolite of pinoxaden (Q1)	317.8	171.2	1	10	23	10
M2 metabolite of pinoxaden (Q2)	317.8	131.1	1	10	57	8

Quantifier (Q1) transitions were used for quantification
Qualifier (Q2) transitions were used for confirmation. Qualifier were monitored but not evaluated.

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Table A 14: Recovery results from method validation of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden, and pinoxaden M2 in OECD medium

Matrix	Analyte	Fortification level (mg a.s./L)*	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
OECD medium	Mesosulfuron-methyl	0.05	96, 98, 100, 99, 101	5	96-101	99	2	-
		0.6	115, 104, 104, 106, 106	5	104-115	107	4	-
		110	85, 99, 92, 87, 95	5	85-99	92	6	-
			Overall	15	85-115	99	8	-
	Mefenpyr-diethyl	0.05	95, 87, 94, 96, 90	5	87-95	92	4	-
		0.6	104, 108, 102, 102, 107	5	102-108	105	3	-
		110	84, 86, 90, 87, 91	5	84-91	88	3	-
			Overall	15	84-108	95	9	-
	Pinoxaden	0.05	97, 102, 105, 103, 90	5	90-105	99	6	-
		0.6	104, 102, 105, 90, 105	5	90-105	101	6	-
		110	82, 87, 90, 80, 85	5	80-90	85	5	-
			Overall	15	80-105	95	9	-
	Pinoxaden M2	1.5 µg/L	98, 91, 93, 95, 94	5	91-98	94	2	-
		18 µg/L	104, 105, 101, 98, 107	5	98-107	103	3	-
		3300 µg/L	83, 88, 89, 84, 84	5	83-89	85	3	-
			Overall	15	83-107	94	8	-

*Limit of quantification, defined by the lowest validated fortification level.
**Residues in control and reagent blank samples were less than 30% of the LOQ
RSD: Relative Standard Deviation

Table A 15: Characteristics for the analytical method used for validation of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and pinoxaden M2 in OECD medium

	Mesosulfuron-methyl, Pinoxaden, Pinoxaden M2 and Mefenpyr-diethyl
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Linearity of Response: Correlation of peak area of different standard solutions (seven levels) with their corresponding concentrations, using a linear regression Calibration range: Mesosulfuron-methyl: Different calibration ranges were evaluated in order to cover the wide concentration range of 0.125 – 20 µg analytical reference item/L with high accuracy. 1. 0.125 – 10 µg analytical reference item/L 2. 0.125 – 20 µg analytical reference item/L Pinoxaden: Different calibration ranges were evaluated in order to cover the wide concentration range of 0.1875 – 90 µg analytical reference item/L with high accuracy. 1. 0.1875 – 3.75 µg analytical reference item/L 2. 7.5 – 90 µg analytical reference item/L Pinoxaden M2: Different calibration ranges were evaluated in order to cover the wide concentration range of 0.125 – 60 µg analytical reference item/L with high accuracy. 1. 0.125 – 2.5 µg analytical reference item/L 2. 0.125 – 60 µg analytical reference item/L Mefenpyr-diethyl: 0.125 – 60 µg analytical reference item/L Correlation Coefficient: r = at least 0.9982 Calibration Curve (linear regression): Mesosulfuron-methyl: 1. y = 324882 * x + 18400, 2. y = 293284 * x + 84875 Pinoxaden: 1. y = 156865 * x + 3552, 2. y = 122976 * x + 401394 Pinoxaden M2: 1. y = 13431 * x + 264, 2. y = 10874 * x + 7042 Mefenpyr-diethyl: y = 104740 * x + 73597, r = 0.9995 For all analytes, calibration data and graphs are presented in the report.
Matrix effects	Not relevant, not tested
Limit of determination (LOD)	Mesosulfuron-methyl: 0.002 µg mesosulfuron-methyl/L Pinoxaden: 0.008 µg pinoxaden/L M2 (metabolite of pinoxaden): 0.08 µg M2/L Mefenpyr-diethyl: 0.06 µg mefenpyr-diethyl/L
Limit of Quantification (LOQ)	Fortification level of nominal 0.05 mg test item/L for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl. For the metabolite of pinoxaden the fortification level of nominal 1.5 µg M2/L.

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and pinoxaden M2 in OECD medium treated with ADM.06001.H.2.B.

A 2.1.1.1.7 Analytical method 7

A 2.1.1.1.7.1 Method validation - Effects on aquatic macrophytes

Comments of zRMS:	The analytical method has been validated for determination of mesosulfuron-methyl, pinoxaden, pinoxaden M2 and mefenpyr-diethyl in test water (20x AAP-Growth Medium) in compliance with SANCO/3029/99 rev.4 and SANTE/2020/12830 rev. 1. The limit of quantification (LOQ) for the test item concentration was determined to be the fortification level of nominal 0.005 mg test item/L for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl. The limit of quantification for the M2 (metabolite of pinoxaden) concentration was determined to be the fortification level of nominal 0.72 µg M2 (metabolite of pinoxaden)/L. The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. The method is acceptable.

Reference:	KCP 5.1.2/07 (also filed under KCP 10.2.1/03)
Report:	ADM.06001.H.2.B: Toxicity to the Aquatic Plant *Lemna gibba* in a Semi-Static Growth Inhibition Test Seidel U. and Mollandin G., 2021c Report no: 140711240, ADAMA reference no: 000105365
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was validated for determination of mesosulfuron-methyl, pinoxaden, pinoxaden M2 and mefenpyr-diethyl in reconstituted water (20x AAP-Growth Medium) coming from ecotoxicological tests with *Lemna gibba* with the test item ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	232-3283	99.4%
Pinoxaden (PNX-400)	243973-20-8	257-3521	99.3%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	98.7%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.82%

Standard Solutions used for Quantification

Stock Solution: The analytical reference item was used to prepare separate stock solutions dissolved in acetonitrile of approximately 1 g analytical reference item/L. Therefore, 20.03 mg of the mesosulfuron-methyl analytical reference item were dissolved in 20 mL acetonitrile, 20.02 mg of the pinoxaden analytical reference item were dissolved in 20 mL acetonitrile containing 0.3% formic acid, 50.29 mg of the M2 (metabolite of pinoxaden) analytical reference item were dissolved in 50 mL acetonitrile and 20.10 mg of the and mefenpyr-diethyl analytical reference item were dissolved in 20 mL acetonitrile.

Standard Solutions:

Appropriate amounts of the stock solutions were diluted with acetonitrile to obtain a mixed analytical standard solution of 10 mg mesosulfuron-methyl/L, 15 mg pinoxaden/L, 10 mg M2 (metabolite of

pinoxaden)/L and 10 mg mefenpyr-diethyl/L. Appropriate amounts of this mixed standard solution were diluted further with test water at pH 4 / acetonitrile (4/1, v/v) to obtain standard solutions in the range from 0.05 to 60 µg mesosulfuron-methyl/L, 0.075 to 90 µg pinoxaden/L, 0.05 to 60 µg M2 (metabolite of pinoxaden)/L and 0.05 to 60 µg mefenpyr-diethyl/L.

Fortified Samples:
50 mg of the test item and 1.5 mL of 1 g M2 (metabolite of pinoxaden)/L stock solution were dissolved in 100 mL acetonitrile to obtain a stock solution of approximately 0.5 g test item/L spiked with 15 mg M2 (metabolite of pinoxaden)/L. Two independent stock solutions were prepared. Appropriate amounts of these stock solutions were diluted with test water at pH 4 / acetonitrile (4/1, v/v) to obtain fortified samples at a level of 0.005, 0.024 and 1 mg test item/L and corresponding the resulting level of 0.15, 0.72 and 30 µg M2/L.

LC-MS/MS chromatographic conditions for Mesosulfuron-methyl, Mefenpyr-diethyl, Pinoxaden and Pinoxaden M2 determination

LC-MS/MS system	Agilent Series 1290 pump and autosampler
Column	Phenomenex Synergi 4 µm Fusion-RP 80A (50x2 mm) For analysis of mesosulfuron-methyl and mefenpyr-diethyl a pre-column Security Guard Cartridge C18 4x2 mm was used.
Column Temperature	40°C
Injection Volume	20 µL
Mobile phases	Eluent A: HPLC-water + 0.1 % HCOOH Eluent B: acetonitrile + 0.1 % HCOOH
Gradient	Time (min) / %Eluent A / % Eluent B / Flow (mL/min) 0 / 95 / 5 / 0.65 2 / 95 / 5 / 0.65 2.5 / 50 / 50 / 0.65 4.5 / 5 / 95 / 0.65 5.2 / 5 / 95 / 0.65 5.3 / 95 / 5 / 0.65 7 / 95 / 5 / 0.65
Run time	7 minutes
MS System	Mass spectrometer API 5500
Ion source	Electrospray ionization (ESI) positive
Curtain gas (CUR): **Nebulizer gas (GS1):**	30 psi 70 psi for pinoxaden and pinoxaden M2 (metabolite of pinoxaden) 85 psi for mesosulfuron-methyl and mefenpyr-diethyl:
Voltage (IS):	5500 V
Heater Temperature (TEM)	350 °C for mesosulfuron-methyl, mefenpyr-diethyl 475 °C for pinoxaden and M2 (metabolite of pinoxaden)
Turbo gas (GS2): **Collision gas (CAD):**	60 psi for pinoxaden and pinoxaden M2 (metabolite of pinoxaden) 70 psi for mesosulfuron-methyl and mefenpyr-diethyl 8
Dwell time:	150 ms for mesosulfuron-methyl, mefenpyr-diethyl 100 ms for pinoxaden and pinoxaden M2 (metabolite of pinoxaden)
MRM mass Transition	

Analyte	Precursor ion [m/z]	Product ion [m/z]	Declustering Potential (DP) [V]	Entrance Potential (EP) [V]	Collision Energy (V)	Collision Exit Potential (CXP) [V]
Mesosulfuron-methyl (Q1)	504.1	182.1	75	12	30	10
Mesosulfuron-methyl (Q2)	504.1	83	75	12	100	40
Mefenpyr-diethyl (Q1)	389.9	327.0	75	2	20	5
Mefenpyr-diethyl (Q2)	389.9	160.0	75	2	47	5
Pinoxaden (Q1)	401.5	317.2	72	10	33	22
Pinoxaden (Q2)	401.5	56.9	72	10	49	8
M2 metabolite of pinoxaden (Q1)	317.8	171.2	101	10	23	10
M2 metabolite of pinoxaden (Q2)	317.8	131.1	101	10	57	8

Quantifier (Q1) transitions were used for quantification
Qualifier (Q2) transitions were used for confirmation. Qualifiers were monitored but not evaluated.

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Table A 16: Recovery results from method validation of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden, and pinoxaden M2 in reconstituted water (20x AAP-Growth Medium)

Matrix	Analyte	Fortification level (mg a.s./L)*	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
20x AAP-Growth Medium	Mesosulfuron-methyl	0.005	109, 113, 111, 110, 99	5	99-113	108	5	-
		0.024	106, 104, 99, 109, 111	5	99-111	106	5	-
		1.0	102, 104, 102, 109, 102	5	102-109	104	3	-
			Overall	15	99-113	106	4	-
	Mefenpyr-diethyl	0.005	107, 82, 94, 104, 53***	4	82-104	97	12	1 outlier
		0.024	81, 110, 102, 92, 89	5	81-110	95	12	-
		1.0	98, 102, 93, 90, 86	5	86-102	94	7	-
			Overall	14	81-110	95	10	-
	Pinoxaden	0.005	115, 108, 111, 109, 110, 107, 107, 111, 103, 105	10	103-111	109	3	-
		0.024	76, 102, 96, 99, 97, 101, 103, 98, 103, 105	10	76-105	98	9	-
		1.0	105, 105, 103, 106, 104, 107, 105, 105, 107, 106	10	103-107	105	1	-
			Overall	30	76-111	104	7	-
	Pinoxaden M2	0.72 µg/L	99, 102, 95, 96, 90, 109, 105, 102, 113, 107	10	90-113	102	7	-
		30 µg/L	99, 102, 93, 90, 100	5	90-102	97	5	-
			Overall	15	90-113	100	7	-

*Limit of quantification, defined by the lowest validated fortification level.
**Residues in control and reagent blank samples were less than 30% of the LOQ
*** value was considered to be an outlier (Grubbs Test, level of significance: P 0.99)
RSD: Relative Standard Deviation

Table A 17: Characteristics for the analytical method used for validation of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and pinoxaden M2 in reconstituted water (20x AAP-Growth Medium)

	Mesosulfuron-methyl, Pinoxaden, Pinoxaden M2 and Mefenpyr-diethyl
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Linearity of Response: Correlation of peak area of different standard solutions (seven levels) with their corresponding concentrations, using a linear regression Calibration range: *Mesosulfuron-methyl:* Different calibration ranges were evaluated in order to cover the wide concentration range of 0.05 – 20 µg analytical reference item/L with high accuracy. 1. 0.05 – 1 µg analytical reference item/L 2. 1 – 20 µg analytical reference item/L Correlation Coefficient: r = at least 0.9983 Calibration Curve (linear regression): 1. y = 204311 * x + 346, 2. y = 211184 * x + 13014, y = 184731 * x - 9216

	Mesosulfuron-methyl, Pinoxaden, Pinoxaden M2 and Mefenpyr-diethyl
	Pinoxaden: Different calibration ranges were evaluated in order to cover the wide concentration range of 0.075 – 30 µg analytical reference item/L with high accuracy. 1. 0.075 – 1.5 µg analytical reference item/L 2. 0.075 – 3.75 µg analytical reference item/L 3. 0.075 – 30 µg analytical reference item/L Correlation Coefficient: r = at least 0.9994 Calibration Curves (linear regression): 1. y = 270357 * x + 1553 2. y = 211162 * x - 5146 3. y = 188168 * x + 17641, y = 223099 * x -+ 53133 *Pinoxaden M2:* 0.2 – 20 µg analytical reference item/L Correlation Coefficient: r = at least 0.9996 Calibration Curves (linear regression): y = 5365 * x + 509, y = 3253 * x – 165 *Mefenpyr-diethyl:* Different calibration ranges were evaluated in order to cover the wide concentration range of 0.05 – 60 µg analytical reference item/L with high accuracy. 1. 0.05 – 1 µg analytical reference item/L 2. 0.05 – 2.5 µg analytical reference item/L 3. 1 – 60 µg analytical reference item/L Correlation Coefficient: r = at least 0.9939 Calibration Curves (linear regression): 1. y = 13187 * x + 67 2. y = 11238 * x - 450 3. y = 10025 * x + 271 For all analytes, calibration data and graphs are presented in the report.
Matrix effects	Not relevant, not tested
Limit of determination (LOD)	Mesosulfuron-methyl: 0.002 µg mesosulfuron-methyl/L Pinoxaden: 0.002 µg pinoxaden/L M2 (metabolite of pinoxaden): 0.130 µg M2/L Mefenpyr-diethyl: 0.036 µg mefenpyr-diethyl/L
Limit of Quantification (LOQ)	Fortification level of nominal 0.005 mg test item/L for mesosulfuron-methyl, pinoxaden and mefenpyr-diethyl. For the metabolite of pinoxaden the fortification level of nominal 0.72 µg M2/L.

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of mesosulfuron-methyl, mefenpyr-diethyl, pinoxaden and pinoxaden M2 in reconstituted water (20x AAP-Growth Medium) treated with ADM.06001.H.2.B.

A 2.1.1.1.8 Analytical method 8

A 2.1.1.1.8.1 Method validation - Chronic oral toxicity test on the honey bee (*Apis mellifera L.*)

Comments of zRMS:	The analytical method is fit for purpose of determining concentrations of mesosulfuron-methyl, pinoxaden and its metabolite M2 and mefenpyr-diethyl in 50 % w/v sucrose solution + 0.1 % Xanthan at LOQ of 1.8 g test item/L for pinoxaden and mesosulfuron-methyl (corresponding to 0.02 g mesosulfuron-methyl (MSF-503)/L (diluted by factor 5000) and corresponding to 0.11 g Pinoxaden (PNX-400)/L (diluted by factor 25000)) and 3.6 g test item/L for Mefenpyr-diethyl (corresponding to 0.14 g Mefenpyr-diethyl (MFN-373)/L (diluted by factor 25000)). The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. No significant amounts of pinoxaden metabolite M2 were detected, therefore no recovery data were measured. The method is acceptable.

Reference: KCP 5.1.2/08 (also filed under KCP 10.3.1.2/01)

Report ADM.06001.H.2.B: Chronic Oral Toxicity Test on the Honey Bee (*Apis*

mellifera L.) in the Laboratory
Report no: 140711136, ADAMA reference no: 000105367

Guideline(s): SANCO/3029/99 rev. 4

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method for determination of mefenpyr-diethyl (MFN-373), mesosulfuron-methyl (MSF-503), pinoxaden (PNX-400) and pinoxaden M2 (PNX-316-HP) in 50 % w/v sucrose solution + 0.1 % Xanthan was validated. Specimen analysis was performed after dilution with a solvent mixture (Acetonitrile/pure water (50/50 v/v) + 0.1 % HCOOH) using LC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 1.8 g test item/L for pinoxaden and mesosulfuron-methyl and 3.6 g test item/L for mefenpyr-diethyl.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	232-3283	99.6 %
Pinoxaden (PNX-400)	243973-20-8	257-3521	99.3%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	98.7%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.82%

Standard Solutions used for Quantification

Solvent Mixture: Acetonitrile/pure water (50/50 v/v) + 0.1 % HCOOH
Stock Solution: The reference items were separately dissolved with acetonitrile to obtain stock solutions of approximately 1 g reference item/L.
Standard Solutions: Aliquots of the stock solutions were combined and diluted with solvent mixture to get mix standard solutions in the range from 1 to 25 mg reference item/L.

Sample Preparation

Sample Preparation: An aliquot of each sample was diluted with solvent mixture.
Matrix: 50 % w/v sucrose solution + 0.1 % Xanthan
Fortification Procedure: The test item was dissolved in matrix (sonicated for 45 minutes) to get fortified samples of about 7.2 g test item/L and 1.8 g test item/L.
Replicates: Five independent replicates per fortification level and two independent replicates of solvent control.

LC-MS/MS chromatographic conditions for Mefenpyr-diethyl, Mesosulfuron-methyl, Pinoxaden and Pinoxaden M2 determination

LC-MS/MS system	Agilent Series 1290 pump and autosampler
Column	Luna Omega 3µmPolar C18 (50 x 3 mm)
Column Temperature	40°C
Injection Volume	5 µL
Mobile phases	Eluent A: HPLC-water + 0.1 % HCOOH Eluent B: acetonitrile + 0.1 % HCOOH

Gradient

Time (min)	%Eluent A	% Eluent B	Flow (mL/min)
0.0	95	5	0.65
2.0	95	5	0.65
2.5	50	50	0.65
4.5	5	95	0.65
5.2	5	95	0.65
5.3	95	5	0.65

	7.5	95	5	0.65	

Mass spectrometric conditions

MS System	API 5500
Detector	MSD, positive mode
Ion Source	5500 V
Temperature	350 °C
Mass Transitions:	
Mefenpyr-diethyl (MFN-373):	390 m/z → 327 m/z (quantifier) 390 m/z → 160 m/z (qualifier)
Mesosulfuron-methyl (MSF-503):	504 m/z →182 m/z (quantifier) 504 m/z → 83 m/z (qualifier)
Pinoxaden (PNX-400):	401 m/z → 317 m/z (quantifier) 401 m/z → 115 m/z (qualifier) 401 m/z → 57 m/z (qualifier)
Pinoxaden M2 (PNX-316-HP):	318 m/z → 171 m/z (quantifier) 318 m/z → 131 m/z (qualifier) 318 m/z → 115 m/z (qualifier)

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Table A 18: Recovery results from method validation of Mefenpyr-diethyl, Mesosulfuron-methyl, Pinoxaden in 50 % w/v sucrose solution + 0.1 % Xanthan

Matrix	Analyte	Fort. level (g test item/L)	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
50 % w/v sucrose solution + 0.1 % Xanthan	Mefenpyr-diethyl (*m/z* 390→327)	3.6*	87, 92, 91, 89, 85	5	85 - 92	89	3	-
		7.20	86, 84, 79, 75, 80	5	75 - 86	81	5	-
		Overall	-	10	75 - 92	85	5	-
	Mesosulfuron-methyl (*m/z* 504→ 182)	1.8*	105, 102, 104, 112, 107	5	102-112	106	4	-
		7.2	106, 98, 104, 107, 105	5	98-107	104	3	-
		Overall	-	10	98-112	105	4	-
	Pinoxaden (*m/z* 401→ 317)	1.8*	100, 103, 108, 111, 112	5	100-112	107	5	-
		7.2	102, 96, 109, 108, 105	5	96-109	104	5	-
		Overall	-	10	96-112	105	5	-

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control and reagent blank samples were less than 30% of the LOQ

Table A 19: Characteristics for the analytical method used for validation of Mefenpyr-diethyl, Mesosulfuron-methyl, Pinoxaden and Pinoxaden M2 residues in 50 % w/v sucrose solution + 0.1 % Xanthan

	Mefenpyr-diethyl, Mesosulfuron-methyl, Pinoxaden and Pinoxaden M2
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Calibration range: 1 to 25 µg reference items/L Calibration Curve (linear regression) and Correlation Coefficient: Mefenpyr-diethyl (MFN-373): y =1552 x – 140; r = 0.9988 Mesosulfuron-methyl (MSF-503): y =140458 x – 34219; r = 0.9992 Pinoxaden (PNX-400): y =55753 x + 6975; r = 0.9995 Pinoxaden M2 (PNX-316-HP): y =12047 x – 1279; r = 0.9995 Calibration data and the graph is presented in the report.
Matrix effects	Not tested, solvent calibration standard used.
Limit of determination	0.2 µg Mefenpyr-diethyl (MFN-373)/L 0.02 µg Mesosulfuron-methyl (MSF-503)/L

	Mefenpyr-diethyl, Mesosulfuron-methyl, Pinoxaden and Pinoxaden M2
(LOD)	0.03 µg Pinoxaden (PNX-400)/L 0.21 µg Pinoxaden M2 (PNX-316-HP)/L
Limit of Quantification (LOQ)	1.8 g test item/L for Pinoxaden and Mesosulfuron-methyl corresponding to 0.02 g Mesosulfuron-methyl (MSF-503)/L (diluted by factor 5000) corresponding to 0.11 g Pinoxaden (PNX-400)/L (diluted by factor 25000) 3.6 g test item/L for Mefenpyr-diethyl corresponding to 0.14 g Mefenpyr-diethyl (MFN-373)/L (diluted by factor 25000)

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of mefenpyr-diethyl, mesosulfuron-methyl, pinoxaden and pinoxaden M2 residues in 50% w/v sucrose solution + 0.1% Xanthan with an LOQ of 1.8 g test item/L for pinoxaden and mesosulfuron-methyl and 3.6 g test item/L for mefenpyr-diethyl.

A 2.1.1.1.9 Analytical method 9

A 2.1.1.1.9.1 Method validation - Honey bee (*Apis mellifera L.*) – 22 d larval chronic toxicity test

Comments of zRMS:	The analytical method has been validated for determination of mesosulfuron-methyl pinoxaden, pinoxaden M2 and mefenpyr-diethyl in test water in compliance with SANCO/3029/99 rev.4 and SANTE/2020/12830 rev. 1 with the limit of quantification (LOQ): mesosulfuron-methyl: 6.37 mg/L; pinoxaden: 33.45 mg/L; pinoxaden M2: 51.49 mg/L; mefenpyr-diethyl: 20.18 mg/L . The mean recovery was within the range 70-110% with a relative standard deviation (RSD) less than to 20%. The method is acceptable.

Reference: KCP 5.1.2/09 (also filed under KCP 10.3.1.3/01)

Report: Effects of ADM.06001.H.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure
Colli M., 2020
Report no: BT138/20 Version 2, ADAMA reference no: 000105368

Guideline(s): SANCO/3029/99 rev. 4

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods
The analytical method was validated for determination of pinoxaden, mesosulfuron-methyl, mefenpyr-diethyl and pinoxaden M2 (PNX-316-HP) in water stock solutions coming from ecotoxicological tests with honey bees with the test item ADM.06001.H.2.B.

Test substance	**CAS No.**	**Batch / Lot No.**	**Purity / content**
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	G141997	99.06 %
Pinoxaden (PNX-400)	243973-20-8	257-3521	99.3%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	99.4%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.5%

Standard Solutions used for Quantification

Solvent: Acetonitrile
Stock Solution: The reference items were separately dissolved with acetonitrile to obtain stock solutions of approximately 1 g reference item/L for and mesosulfuron-methyl and of approximately 2 g reference item/L for mefenpyr-diethyl. Aliquots of the stock solutions were combined and diluted with acetonitrile to get mix standard solutions containing of 6357.5 µg/L pinoxaden, 1029.8 µg/L pinoxaden M2, 1237.0 µg/L mesosulfuron-methyl and 3701.6 µg/L mefenpyr-diethyl.

Standard Solutions: Aliquots of the mix stock solution was further diluted with acetonitrile to get 5 level of standard solutions in the range of: 63.6 to 635.7 µg /L for pinoxaden, 10.3 to 103.0 µg /L for pinoxaden M2, 12.4 – 123.7 µg/L for mesosulfuron-methyl and 37.2 to 370.2 µg/L for mefenpyr-diethyl.

LC-MS/MS chromatographic conditions for Pinoxaden, Mesosulfuron-methyl and Mefenpyr-diethyl determination

LC-MS/MS system	Agilent Series 1290 pump and autosampler
Column	Agilent Zorbax Eclipse Plus C 18 RRHD, 3 x 50 mm, 1.8 µm
Column Temperature	40°C
Injection Volume	0.5 µL
Mobile phases	Eluent A: HPLC-water + 0.1 % HCOOH Eluent B: acetonitrile + 0.1 % HCOOH
Gradient	Time (min) / %Eluent A / % Eluent B / Flow (mL/min) 4.5 / 10 / 90 / 0.60 5.5 / 10 / 90 / 0.60 5.6 / 80 / 20 / 0.60
Run time	7 minutes
Retention time	Pinoxaden M2: 2.3 minutes Pinoxaden: 3.4 minutes Mesosulfuron-methyl: 2.3 minutes Mefenpyr-diethyl: 3.9 minutes
MS System	6495a Triple Quadrupole Spectrometer
Polarity	positive

Transition	**Precursor ion MS1**	**Product ion MS2**	**Cycle time / Dwell (ms)**	**Fragmentor (V)**	**Collision Energy (V)**
Pinoxaden					
Quantifier (Q1)	401	317.2	500	380	20
Qualifier (Q2)	401	56.9			50
Mesosulfuron-methyl					
Quantifier (Q1)	504.1	182.1	500	380	24
Qualifier (Q2)	504.1	83			60
Mefenpyr-diethyl					
Quantifier (Q1)	373	327	500	380	10
Qualifier (Q2)	373	159.6			30
Pinoxaden M2					
Quantifier (Q1)	317.1	171.2	200	380	10
Qualifier (Q2)	317.1	130.9			50

Quantifier (Q1) transitions were used for quantification
Qualifier (Q2) transitions were used for confirmation

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Table A 20: Recovery results from method validation of Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2 in water

Matrix	Analyte	Fort. level (mg/L)	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
Water	Pinoxaden (Q1 401.0→ 317.2)	33.45*	108, 101, 102, 102, 101	5	101-108	103	3	-
		1990	97, 105, 102, 98, 98	5	97-105	100	3	-
	Pinoxaden (Q2 401.0→ 56.9)	33.45*	98, 94, 95, 105, 91	5	91-105	97	6	-
	Mesosulfuron-methyl (Q1 504.1→ 182.1)	6.37*	103, 103, 100, 97, 97	5	97-103	100	3	-
		374.8	101, 95, 98, 100, 100	5	95-101	99	2	-
	Mesosulfuron-methyl (Q2 504.1→ 83)	6.37*	93, 97, 95, 103, 95	5	93-103	96	4	-
	Mefenpyr-diethyl (Q1 373→327)	20.18	92, 93, 100, 99, 92	5	92-100	95	4	-
		1190	97, 96, 96, 90, 94	5	90-97	95	3	-
	Mefenpyr-diethyl (Q2 373→159.6)	20.18	96, 95, 97, 101, 99	5	95-101	98	3	-
	Pinoxaden M2 (Q1 317.1→ 171.2)	51.49 µg/L	89, 89, 91, 89, 89	5	89-91	90	1	-
		154.47	91, 92, 93, 92, 93	5	91-93	92	1	-
	Pinoxaden M2 (Q2 317.1→ 130.9)	51.49 µg/L	97, 97, 99, 99, 96	5	96-99	97	1	-

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control and reagent blank samples were less than 30% of the LOQ

Table A 21: Characteristics for the analytical method used for validation of Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2 in water

	Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Calibration range: Pinoxaden: 63.6 to 635.7 µg/L Mesosulfuron-methyl: 12.4 – 123.7 µg/L Mefenpyr-diethyl: 37.2 - 370.2 µg/L Pinoxaden M2: 10.30 – 102.98 µg/L Calibration Curve (linear regression) for Q1: Pinoxaden: y =299.781590x + 4483.992; r = 0.9994 Mesosulfuron-methyl: y =23.556333 x –4.413270, r=0.9986 Mefenpyr-diethyl: y =32.046932 x – 81.644734, r=0.9977 Pinoxaden M2: y =49.139234x +.99.038336, r=0.9939 Calibration Curve (linear regression) for Q2: Pinoxaden: y =58.4762x + 1349.1007; r = 0.9979 Mesosulfuron-methyl: y = 7.768648 x – 4.652441, r=0.9985

	Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2
	Mefenpyr-diethyl: y = 5.203841x – 26.444525, r=0.9988 Pinoxaden M2: y =50.760216 x + 4.334453 Calibration data and graphs are presented in the report.
Matrix effects	Matrix effects at recovery levels are negligible (<20%), therefore the solutions to be analysed were prepared in solvent.
Limit of determination (LOD)	63.6 µg/L Pinoxaden 12.4 µg/L Mesosulfuron-methyl 37.0 µg/L Mefenpyr-diethyl 10.30 µg/L Pinoxaden M2
Limit of Quantification (LOQ)	33.45 mg/L Pinoxaden 6.37 mg/L Mesosulfuron-methyl 20.18 mg/L Mefenpyr-diethyl 51.49 µg/L Pinoxaden M2

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of pinoxaden, mesosulfuron-methyl, mefenpyr-diethyl and pinoxaden M2 in water solutions treated with ADM.06001.H.2.B.

A 2.1.1.1.10 Analytical method 10

A 2.1.1.1.10.1 Method validation - Chronic toxicity to the earthworm *Eisenia andrei* (Oligochaeta: Lumbri-cidae) in an artificial soil substrate

Comments of zRMS:	The analytical method is fit for purpose of determining concentrations of mesosulfuron-methyl in soil at LOQ of 10 mg test item/kg dry soil (10.6 µg /L). The method is acceptable.

Reference: KCP 5.1.2/10 (also filed under KCP 10.3.1.3/01)

Report: ADM.06001.H.2.B: Determination of chronic toxicity to the earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae) in an artificial soil substrate
Straube D. and Gourlay V., 2021
Report no: 140711022, ADAMA reference no: 000105375

Guideline(s): SANCO/3029/99 rev. 4

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods
The analytical method was validated for determination of mesosulfuron-methyl in soil coming from ecotoxicological tests with earthworm with the test item ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	232-3283	99.6 %

Standard Solutions used for Quantification
Standard Solvent Mixture: Matrix/Acetonitrile/pure water (50/25/25, v/v/v)
Stock Solution: The reference item was dissolved with acetonitrile to obtain a stock solution of approximately 1 g reference item/L.

Standard Solutions: Aliquots of the stock solution were diluted with solvent mixture to get standard solutions in the range of 1 to 100 µg reference item/L.

Fortification Procedure:

A stock solution with the test item (182.94 mg) was dissolved in pure water (25 mL) to a concentration of 7317.6 mg/L. An application solution was prepared via dilution with pure water to a concentration of 731.76 mg/L.

The test solutions were spiked to soil matrix in order to get fortified samples of about 10 and 1110 mg test item/ dry kg soil. The fortified samples were then agitated for 1 h.

Replicates:

Five independent replicates per fortification level, two independent replicates of matrix control (spiked with pure water).

Sample Extraction

Extraction Solvent Mixture: Acetonitrile/pure water (80/20, v/v)

Extraction Procedure: About 2 g aliquots (equivalent dry soil) of each sample (test, fortified or blank) were extracted three times with 8 mL of acetonitrile/pure water (80/20, v/v). The three extraction solutions were combined and the volume was determined.

Replicates: Two replicates per test concentration (Conc. 1, Conc. 8, Control I-IV and Control V-VIII)
Five independent replicates per fortification level
Two independent replicates of matrix control

Sample Preparation

Dilution Solvent Mixture: Acetonitrile/pure water (50/50, v/v)

Sample Preparation: An aliquot of each extraction sample (Conc. 1, Conc. 8, Control I-IV, Control V-VIII, fortified or matrix blank) was diluted with solvent mixture. The dilution factor corresponded to either 2 for the low rates and 20 for high rates. Control and matrix blank extracts were not diluted.

Replicates: Two replicates per test concentration (Conc. 1, Conc. 8, Control I-IV and Control V-VIII)
Five independent replicates per fortification level
Two independent replicates of matrix control

HPLC chromatographic conditions for Mesosulfuron-methyl determination

LC-MS system	Agilent Series 1200 pump and autosampler
Column	PerfectSil 120 ODS-2 (125 * 3 mm; 5 µm)
Column Temperature	20°C
Injection Volume	20 µL
Mobile phases	Eluent A:30 % HPLC water + 5 mM NH4CH3COO Eluent B: 70 % acetonitrile + 5 mM NH4CH3COO Isocratic
MS System	API 3200, Sciex
Polarity	ESI positive
IS	2000 V
Temperature	550 °C
Run time	7.0 min
Mass Transitions:	Quantifier: 504 → 182 amu Qualifier: 504 → 83 amu

Results and discussions
Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Table A 22: Recovery results from method validation of Mesosulfuron-methyl in soil

Matrix	Analyte	Fort. level (g/kg dry soil)	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
Soil	Mesosulfuron-methyl (504 → 182 amu)	10*	74, 79, 63, 72, 80	5	63-80	74	9	-
		1110	85, 99, 91, 90, 90	5	85-99	91	6	-

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control and reagent blank samples were less than 30% of the LOQ

Table A 23: Characteristics for the analytical method used for validation of mesosulfuron-methyl in soil

	Mesosulfuron-methyl
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Calibration range: Mesosulfuron-methyl: 1.0 to 100 µg mesosulfuron-methyl/L (8 levels) Calibration Curve (linear regression): Mesosulfuron-methyl: y = 5162 x + 2261, r=0.9962 Calibration data and the graph is presented in the report.
Matrix effects	Not tested
Limit of Quantification (LOQ)	10 mg mesosulfuron-methyl /kg dry soil
Limit of determination (LOD)	0.3 µg mesosulfuron-methyl/L

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of mesosulfuron-methyl in soil samples treated with ADM.06001.H.2.B.

A 2.1.1.1.11 Analytical method 11

A 2.1.1.1.11.1 Method validation - Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test

Comments of zRMS:	The analytical method is fit for purpose of determining concentrations of mesosulfuron-methyl, pinoxaden and its metabolite M2 and mefenpyr-diethyl in water at LOQ of 11 mg /L for mesosulfuron-methyl, LOQ of 60 mg /L for pinoxaden and LOQ of 36 mg/L for mefenpyr-diethyl. The method is acceptable.

Reference: KCP 5.1.2/11 (also filed under KCP 10.6.2/01)

Report: ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test
Spatz B. and Kowalczyk F., 2021a
Report no: 140711086, ADAMA reference no: 000105379

Guideline(s): SANCO/3029/99 rev. 4

Deviations: None

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method was validated for determination of mesosulfuron-methyl, pinoxaden and its metabolite M2 and mefenpyr-diethyl in an aqueous test item spraying solution coming from ecotoxicological tests with non-target plants with the test item ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	G1016677	98.23 %
Pinoxaden (PNX-400)	243973-20-8	257-3521	98.8%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	98.7%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.82%

Standard Solutions used for Quantification

Standard Solvent Mixture: Acetonitrile/pure water (50/50 v/v) + 0.1 % HCOOH

Stock Solution: The reference items were separately dissolved with acetonitrile to obtain stock solutions of approximately 1 g reference item/L.

Standard Solutions: Aliquots of the stock solutions were combined and diluted with solvent mixture to get standard solutions in the range from 0.5 to 20 µg/L for each reference item.

Sample Preparation:

One specimen of the stock solution sample and control solution sample taken from the biological study were made to the mark (25 mL) with acetonitrile. These solutions were then diluted with solvent mixture to match calibration range. First dilution step was performed while solutions were stirring.

Fortification Procedure:

The test item was dissolved in pure water to get fortified samples of about 6.0 g test item/L and 1.0 g test item/L.

Replicates:

Five independent replicates per fortification level Two independent replicates of solvent control

LC-MS/MS chromatographic conditions for Pinoxaden, Pinoxaden M2, Mesosulfuron-methyl and Mefenpyr-diethyl determination

LC-MS/MS system	Agilent Series 1290 pump and autosampler
Column	Luna Omega Polar C18 (50 x 3 mm, 3 µm)
Column Temperature	40°C
Injection Volume	5 µL
Mobile phases	Eluent A: HPLC-water + 0.1 % HCOOH Eluent B: acetonitrile + 0.1 % HCOOH
Gradient	

Time (min)	%Eluent A	% Eluent B	Flow (mL/min)
Initial	95	5	0.65
2.0	95	5	0.65
2.5	50	80	0.65
4.5	5	95	0.65
5.2	5	95	0.65
5.3	95	5	0.65
7.0	95	5	0.65

Mass Spectrometer	API 5500
Polarity	Positive mode
Ion Source [V] **Temperature [°C]** **Scan Type**	5500 350 MRM
Mefenpyr-diethyl (MFN-373)	390 m/z → 327 m/z (quantifier) 390 m/z → 160 m/z (qualifier)
Mesosulfuron-methyl (MSF-503)	504 m/z → 182 m/z (quantifier) 504 m/z → 83 m/z (qualifier)
Pinoxaden (PNX-400)	401 m/z → 317 m/z (quantifier) 401 m/z → 115 m/z (qualifier)
Pinoxaden M2 (PNX-316-HP)	318 m/z → 171 m/z (quantifier) 318 m/z → 131 m/z (qualifier)

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).
The pinoxaden metabolite M2 was nor detected in the samples, therefore no recovery data were measured.

Table A 24: Recovery results from method validation of Pinoxaden, Mesosulfuron-methyl and Mefenpyr-diethyl in water

Matrix	Analyte	Fort. level (g/L)	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
Water	Mesosulfuron-methyl	1*	107, 105, 104, 114, 112	5	104-114	108	4	-
		6	113, 101, 103, 119, 101	5	101-119	108	7	-
		Overall		10	101-119	108	5	-
	Pinoxaden	1	103, 101, 107, 102, 109	5	101-109	104	3	-
		6	91, 103, 97, 113, 106	5	91-113	104	8	-
		Overall		10	93-103	103	6	-
	Mefenpyr-diethyl	1	98, 98, 110, 106, 99	5	98-110	102	5	-
		6	98, 101, 100, 100, 91	5	91-101	98	4	-
		Overall		10	95-101	100	5	-

*Limit of quantification, defined by the lowest validated fortification level
**Residues in analytical solvent control samples were < LOD

Table A 25: Characteristics for the analytical method used for validation of Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2 in water

	Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Calibration range: 0.5 to 20 µg mesosulfuron-methyl/L 0.5 to 20 µg pinoxaden/L 0.5 to 20 µg mefenpyr-diethyl/L Calibration Curve (linear regression): Mesosulfuron-methyl: y = 36576 x – 7085, r=0.9995 Pinoxaden: y = 18993 x + 10438, r=0.9971 Mefenpyr-diethyl: y = 11281 x – 796, r=0.9999 Calibration data and graphs are presented in the report.

	Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2
Matrix effects	Not tested, not relevant
Limit of determination (LOD)	0.01 µg mesosulfuron-methyl /L 0.02 µg pinoxaden /L 0.08 µg mefenpyr-diethyl /L
Limit of Quantification (LOQ)	1.0 g test item /L corresponding to 4.8 µg mesosulfuron-methyl after dilution by factor 2500 corresponding to 5.0 µg pinoxaden after dilution by factor 12500 corresponding to 3.0 µg mefenpyr-diethyl after dilution by factor 12500

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of pinoxaden, mesosulfuron-methyl and mefenpyr-diethyl in water solutions treated with ADM.06001.H.2.B.

A 2.1.1.1.11.2 Method validation - Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test

Comments of zRMS:	The analytical method is fit for purpose of determining concentrations of mesosulfuron-methyl, pinoxaden and its metabolite M2 and mefenpyr-diethyl in water at LOQ of 1.0 g test item /L (corresponding to 4.8 µg mesosulfuron-methyl after dilution by factor 2500, corresponding to 5.0 µg pinoxaden after dilution by factor 12500 and corresponding to 3.0 µg mefenpyr-diethyl after dilution by factor 12500). The method is acceptable.

Reference:	KCP 5.1.2/12 (also filed under KCP 10.6.2/02)
Report	ADM.06001.H.2.B: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Spatz B. and Kowalczyk F., 2021b Report no: 140711087, ADAMA reference no: 000105380
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods
The analytical method was validated for determination of mesosulfuron-methyl, pinoxaden and its metabolite M2 and mefenpyr-diethyl in an aqueous test item spraying solution coming from ecotoxicological tests with non-target plants with the test item ADM.06001.H.2.B.

Test substance	CAS No.	Batch / Lot No.	Purity / content
ADM.06001.H.2.B	-	A8001	Content of a.s.: mesosulfuronmethyl:12.1g/L, pinoxaden: 61.6 g/L, mefenpyr-diethyl: 36.6 g/L,
Mesosulfuron-methyl (MSF-503)	208465-21-8	G1016677	98.23%
Mesosulfuron-methyl (MSF-503)	208465-21-8	232-3283	99.6%
Pinoxaden (PNX-400)	243973-20-8	257-3521	99.3%
Pinoxaden M2 (PNX-316-HP)	314020-44-5	239-3354	98.7%
Mefenpyr-diethyl (MFN-373)	135590-91-9	114-2079	99.82%

Standard Solutions used for Quantification
Standard Solvent Mixture:
1st Application (test with all species except *Solanum lycopersicum*): Acetonitrile/pure water (50/50 v/v) + 0.1 % HCOOH

2nd Application (test with *Solanum lycopersicum*): Acetonitrile/pure water (50/50 v/v)
Stock Solution: The reference items were separately dissolved with acetonitrile to obtain stock solutions of approximately 1 g reference item/L.
Standard Solutions: Aliquots of the stock solutions were combined and diluted with solvent mixture to get standard solutions in the range from 0.5 to 20 µg/L for each reference item.

Sample Preparation:
An aliquot of each sample taken from the first application was diluted with solvent mixture. One specimen of the stock solution sample and control solution sample taken from the 2nd application were made to the mark (25 mL) with acetonitrile. These solutions were then diluted with solvent mixture to match calibration range. First dilution step was performed while solutions were stirring.
Fortification Procedure:
The test item was dissolved in pure water to get fortified samples of about 6.0 g test item/L and 1.0 g test item/L.
Replicates:
At least five independent replicates per fortification level, At least two independent replicates of solvent control.

LC-MS/MS chromatographic conditions for Pinoxaden, Pinoxaden M2, Mesosulfuron-methyl and Mefenpyr-diethyl determination

<table>
<tr><td>LC-MS/MS system</td><td>Agilent Series 1290 pump and autosampler</td></tr>
<tr><td>Column</td><td>Luna Omega Polar C18 (50 x 3 mm, 3 µm)</td></tr>
<tr><td>Column Temperature</td><td>40°C</td></tr>
<tr><td>Injection Volume</td><td>5 µL</td></tr>
<tr><td>Mobile phases</td><td>Eluent A: HPLC-water + 0.1 % HCOOH
Eluent B: acetonitrile + 0.1 % HCOOH</td></tr>
<tr><td>Gradient</td><td>
<table>
<tr><td>Time (min)</td><td>%Eluent A</td><td>% Eluent B</td><td>Flow (mL/min)</td></tr>
<tr><td>Initial</td><td>95</td><td>5</td><td>0.65</td></tr>
<tr><td>2.0</td><td>95</td><td>5</td><td>0.65</td></tr>
<tr><td>2.5</td><td>50</td><td>80</td><td>0.65</td></tr>
<tr><td>4.5</td><td>5</td><td>95</td><td>0.65</td></tr>
<tr><td>5.2</td><td>5</td><td>95</td><td>0.65</td></tr>
<tr><td>5.3</td><td>95</td><td>5</td><td>0.65</td></tr>
<tr><td>7.0</td><td>95</td><td>5</td><td>0.65</td></tr>
</table>
</td></tr>
<tr><td>Mass Spectrometer</td><td>API 5500</td></tr>
<tr><td>Polarity</td><td>Positive mode</td></tr>
<tr><td>Ion Source [V]
Temperature [°C]
Scan Type</td><td>5500
350
MRM</td></tr>
<tr><td>Mefenpyr-diethyl (MFN-373)</td><td>390 m/z → 327 m/z (quantifier)
390 m/z → 160 m/z (qualifier)</td></tr>
<tr><td>Mesosulfuron-methyl (MSF-503)</td><td>504 m/z → 182 m/z (quantifier)
504 m/z → 83 m/z (qualifier)</td></tr>
<tr><td>Pinoxaden (PNX-400)</td><td>401 m/z → 317 m/z (quantifier)
401 m/z → 115 m/z (qualifier)</td></tr>
<tr><td>Pinoxaden M2 (PNX-316-HP)</td><td>318 m/z → 171 m/z (quantifier)
318 m/z → 131 m/z (qualifier)</td></tr>
</table>

Results and discussions
Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).
The pinoxaden metabolite M2 was nor detected in the samples, therefore no recovery data were measured.

Table A 26: Recovery results from method validation of Pinoxaden, Mesosulfuron-methyl and Mefenpyr-diethyl in water

Matrix	Analyte	Fort. level (g/L)	Recovery (%)**	n	Recovery Range (%)	Mean Recovery (%)	RSD (%)	Comments
Water	Mesosulfuron-methyl	1*	107, 105, 104, 114, 112	5	104-114	108	4	-
		6	113, 101, 106, 119 101, 100, 94, 78, 91, 93	10	78-119	100	11	-
		Overall		15	78-119	103	10	-
	Pinoxaden	1	103, 101, 107, 102, 109	5	101-109	104	3	-
		6	95, 92, 76, 91, 96, 91, 103, 97, 113, 106	10	76-113	96	10	-
		Overall		15	76-113	99	9	-
	Mefenpyr-diethyl	1	98, 98, 110, 106, 99	5	98-110	102	5	-
		6	100, 102, 99, 94, 103, 98, 101, 100, 100, 91	10	91-103	100	3	-
		Overall		15	91-110	100	5	-

*Limit of quantification, defined by the lowest validated fortification level
**Residues in analytical solvent control samples were < LOD

Table A 27: Characteristics for the analytical method used for validation of Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2 in water

	Pinoxaden, Mesosulfuron-methyl, Mefenpyr-diethyl and Pinoxaden M2
Specificity and Selectivity	LC-MS/MS is considered to be highly specific and therefore, no further confirmatory technique is required. No interference above 30% of target analytes was observed.
Calibration range Calibration (type, number of data points)	Calibration range: 1st application: 1.0 to 20 µg reference item/L 2nd application: 0.5 to 20 µg reference item/L Calibration Curve (linear regression): Mesosulfuron-methyl: y = 36576 x – 7085, r=0.9995 Pinoxaden: y = 18993 x + 10438, r=0.9921 Mefenpyr-diethyl: y = 11281 x – 796, r=0.9998 Calibration data and graphs are presented in the report.
Matrix effects	Not tested, not relevant
Limit of determination (LOD)	0.01 µg mesosulfuron-methyl /L 0.02 µg pinoxaden /L 0.08 µg mefenpyr-diethyl /L
Limit of Quantification (LOQ)	1.0 g test item /L corresponding to 4.8 µg mesosulfuron-methyl after dilution by factor 2500 corresponding to 5.0 µg pinoxaden after dilution by factor 12500 corresponding to 3.0 µg mefenpyr-diethyl after dilution by factor 12500

Conclusion
The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of pinoxaden, mesosulfuron-methyl and mefenpyr-diethyl in water solutions treated with ADM.06001.H.2.B.

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

In relation to residues in the commodities for which use of this product is proposed (winter and spring wheat, rye, triticale) and in appropriate animal matrices and environmental samples, the applicant has

full access to the appropriate monitoring methods found acceptable in the DARs (or to studies which have been deemed equivalent) and the conclusions made on the basis of those studies. No further consideration is therefore necessary in this evaluation.

Exception: A method validation study for the determination of residues of mesosulfuron-methyl in human urine was performed to fulfil the data requirements. The summary of this study is presented later under point A 2.1.2.6.

For validations of analytical methods used for data generation for risk assessment, please refer to A 2.1.1.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.1.2.3 Description of methods for the analysis of mesosulfuron-methyl in soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of methods for the analysis of mesosulfuron-methyl in water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of methods for the analysis of mesosulfuron-methyl in air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of methods for the analysis of mesosulfuron-methyl residues in body fluids and tissues (KCP 5.2)

A 2.1.2.6.1 Analytical method 1

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	The analytical method RES-00291 has been validated for determination of residues of mesosulfuron-methyl in human urine with LOQ of 0.01 mg/L in compliance with SANTE/2020/12830 rev. 1. For mesosulfuron-methyl the mean recovery value was in the range 70 – 110% with a relative standard deviation of ≤ 20%. The method is acceptable.

Reference:	KCP 5.2/01
Report:	Validation of an Analytical Method for the Determination of Residues of Mesosulfuron-methyl in human urine by LC-MS/MS Final Report Amendment No. 1 Watson G., 2021 Report no: ES-00291, ADAMA reference no: 000106703
Guideline(s):	OECD ENV/JM/MONO(2007)17 SANCO/3029/99 rev.4 (11/07/00) SANCO/825/00 rev. 8.1 EPA OPPTS 860.1340 (1996)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method (RES-00291) was validated for determination of residues of mesosulfuron-methyl in human urine. The analytical method involved dilution of the sample with acetonitrile/water (50/50, v/v) prior to quantification by LC-MS/MS. A limit of quantification (LOQ) of 0.01 mg/L was validated.

Test substance	CAS No.	Batch / Lot No.	Purity / content
Mesosulfuron-methyl	208465-21-8	179-2761	99.5%

Standard Solutions used for Quantification

Stock Solution: A mesosulfuron-methyl stock solution was prepared in acetonitrile at a concentration of 500 µg/mL by dissolving 10.14 mg in 20.28 mL of solvent with the aid of an ultrasonic bath. The stock solution was further diluted for the preparation of fortification, intermediate and calibration standards.

Standard Solutions:

Matrix matched calibration standard solutions were prepared by serial dilution of the appropriate intermediate solution using control matrix final sample. Appropriate amounts were diluted further to obtain standard solutions in the range from 0.000015 to 0.01 µg/mL.

A solvent calibration standard solution for matrix effect assessment was prepared by serial dilution of the appropriate intermediate solution using acetonitrile/water (50/50, v/v) to obtain a concentration of 0.0005µg/mL.

Standard Solutions for Stability Testing

A new mesosulfuron-methyl stock solution was prepared in acetonitrile at a concentration of 500µg/mL by dissolving 10.04 mg in 20.08 mL of solvent with the aid of an ultrasonic bath. The new stock solution was further diluted to prepare fresh matrix matched calibration standards for extract stability testing.

The original 0.125 µg/mL and new 0.125 µg/mL solvent intermediate standards were diluted with acetonitrile/water (50/50, v/v) for standard stability assessment.

Extraction

5.0 mL of the human urine sample was accurately dispensed in to a 15 mL polypropylene centrifuge tube. Procedural recovery specimens were fortified at this stage by addition of the appropriate fortification solution to receive a 0.01 mg/mL fortification level. The centrifuge tube was mixed after fortification. A 50 µL aliquot of sample was mixed with 950 µL of acetonitrile/water (50/50, v/v) and analysed by LC-MS/MS. The final sample concentration was 0.05 mL/mL.

LC-MS/MS chromatographic conditions for Mesosulfuron-methyl determination

LC-MS/MS system	AB Sciex 5500 QTrap Mass Spectrometer with an Agilent 1100 Binary HPLC Pump, Agilent 1100 Degasser, Agilent 1100 Column Oven, CTC Analytics HTS PAL Autosampler and a Peak Scientific Genius 1024 Gas Generator				
Column	Phenomenex Kinetex C18, 50 x 4.6 mm, 2.6 µm particle size				
Column Temperature	30°C				
Injection Volume	2 µL				
Mobile phases	Eluent A: water Eluent B: acetonitrile				
Gradient	Time (min) / %Eluent A / % Eluent B / Flow (mL/min) 0.00 / 80 / 20 / 0.5 4.50 / 65 / 65 / 0.5 5.50 / 65 / 65 / 0.5 5.51 / 0 / 100 / 0.5 7.00 / 0 / 100 / 0.5 7.01 / 80 / 20 / 0.5 9.00 / 80 / 20 / 0.5				
Retention time	4.6 minutes				
Ionisation mode	Turbo Ion Spray (Electrospray)				
Polarity	Positive				
Curtain Gas **CAD Gas** **Gas 1** **Gas 2**	45 (arbitrary units) Medium (arbitrary units) 50 (arbitrary units) 50 (arbitrary units)				
Source temperature	600°C				
Spray Voltage:	5500 V				
Entrance Potential	12 V				
Declustering Potential	65 V				
Mass Transitions	Ions monitored (++)	Dwell time (msec)	Collision Energy	Cell Exit Potential	Primary / Confirmatory
Mesosulfuron-methyl	504.0 → 182.0	100V	33V	10V	Primary
	504.0 → 83.0	100	80V	10V	Confirmatory

Results and discussions

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/825/00 rev. 8.1, (70 - 110 % mean recovery, 20 % RSD).

Table A 28: Recovery results from method validation of mesosulfuron-methyl in human urine

Matrix	Fortification level (mg/L)*	Recovery (%)	Mean Recovery (%)	RSD (%)	n	Recovery Range (%)	Comments
Mesosulfuron-methyl with m/z 504.0 → 182.0 (Primary)							
Reagent Blank**	-	-	-	-	1		-
Urine	Control**	-	-		2		-
	0.01*	93, 96, 96, 94, 94	95	1.9	5	93-96	-
Mesosulfuron-methyl with m/z 504.0 → 83.0 (Confirmatory)							
Reagent Blank**	-	-	-	-	1		-
Urine	Control**	-	-		2		-
	0.01*	91, 97, 97, 97, 92	95	3.1	5	91-97	-

*Limit of quantification, defined by the lowest validated fortification level.

**Residues in control and reagent blank samples were less than 30% of the LOQ
RSD: Relative Standard Deviation

Table A 29: Characteristics for the analytical method used for validation of mesosulfuron-methyl in human urine

	Mesosulfuron-methyl
Specificity and Selectivity	Final determination of mesosulfuron-methyl was conducted by LC-MS/MS monitoring transitions 504.0 → 182.0 *m/z* (primary) and 504.0 → 83.0 *m/z* (confirmatory). A reagent blank and two control specimens were extracted and analysed to investigate the presence of mesosulfuron-methyl and/or matrix interference at the retention times of the analyte. The selectivity of the method was demonstrated as no matrix interferences or residues of mesosulfuron-methyl were observed at or above 30% of the LOQ in the reagent blank sample or the control samples. The analytical method validated achieves a high level of specificity. Thus, an additional confirmatory method is not required.
Calibration range Calibration (type, number of data points)	Detector linearity was assessed on a run by run basis by constructing a calibration curve of peak area versus analyte concentration. Linear regression at 6 different concentrations ranging from 0.00015 µg/mL to 0.01 µg/mL was used. This is equivalent to 30% of the LOQ (0.003 mg/L) to 0.2 mg/L. Correlation coefficients (r) above 0.995 were obtained for mesosulfuron-methyl. $y = 178049960.8702x + 6306.2050$, $r^2 = 0.9974$, primary $y = 51976264.9762x + 809.6097$, $r^2 = 0.9970$, confirmatory Calibration data and graphs are presented in the report.
Matrix effects	No significant (i.e. >20%) matrix effects were observed for mesosulfuron-methyl but matrix matched standards were used for quantification.
Limit of determination (LOD)	The limit of detection was confirmed to be less than 30% of the LOQ as demonstrated by the response of the bottom calibration standard (equivalent to 30% of the LOQ) which was greater than three times the signal to noise for mesosulfuron-methyl for both mass transitions monitored.
Limit of Quantification (LOQ)	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/L in human urine.
Stability in Final Sample Extract	Extract stability was assessed by re-injection of the LOQ recoveries using freshly prepared calibration standards after 9 days refrigerated storage. For mesosulfuron-methyl the mean recovery value was in the range 70 – 110% with a relative standard deviation of ≤ 20% and was within ± 20% of the original value. Extracts are therefore deemed to be stable for at least 9 days when stored refrigerated.
Stability of standard solutions	Stability of mesosulfuron-methyl standard solutions was assessed by comparing a freshly prepared 0.125 µg/mL standard solution to a 0.125 µg/mL standard solution that had been stored refrigerated for 11 days. Both standards were prepared in acetonitrile/water (50/50, v/v) and both were diluted 250-fold with acetonitrile/water (50/50, v/v) prior to LC-MS/MS analysis. Each standard was injected in triplicate. Stability of the 0.125 µg/mL intermediate standard solution was assessed as this standard is used to prepare the LOQ equivalent matrix matched calibration standard. Solvent calibration standards were shown to be stable for 11 days when stored refrigerated which covers the time standards used in the study.

Conclusion

The applicability of the used method for the analysis of residues of mesosulfuron-methyl in human urine samples was tested. The specimen extracts were analysed using liquid chromatography with mass selective detection (LC-MS/MS).
The analytical method was found to be valid for the determination of mesosulfuron-methyl in human urine with an LOQ of 0.01 mg/L. The validation of the method met the criteria detailed in SANCO/825/00 rev. 8.1.

Syngenta clarify studies or analysis in water are included with confirmatory data submitted to all MSs, RMS Austria launched a public consultation in Q2 2022 and the studies are evaluated in RAR Addendum 1 to Volume 3. The studies were submitted in some Syngenta product dossiers already, but not finally evaluated.

Comments of zRMS:	Analytical method GRM017.06A has been acceptably validated according to the SANE/2020/12830 rev.1 for the determination of pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in groundwater and surface water with the limit of quantification (LOQ) of 0.05 µg/L.

	The mean recovery values were in the range 70 – 110% with a relative standard deviation of ≤ 20%. The method is acceptable.

Reference:	KCP 5.2/02
Report	Crook, S., Langridge, G., McCarthy, I. (2015). Pinoxaden - Residue Method GRM017.06A for the Determination of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 in Water by Direct Injection LC-MS/MS Analysis. Syngenta Analytical Method (Report Number GRM017.06A). Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK Syngenta File No. NOA407855_10321; ; VV-132582
Reference:	KCP 5.2/03
Report	Langridge G. (2015a). Pinoxaden - Validation of Draft Residue Method GRM017.06A for the Determination of Pinoxaden and Its Metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in Water. CEMAS Report Number CEMR-6750-REG. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Syngenta File No. NOA407855_10320; VV-414220
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000) Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012
Deviations:	No
GLP:	Validation: Yes; Method: No
Acceptability:	Yes

Principle of the Method

In summary, water is extracted by solid phase extraction before analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS) for pinoxaden, NOA407854 (M2), NOA447204 (M3), SYN504574 (M11), SYN546105, SYN546106, SYN546107, SYN546108. The limit of quantification (LOQ) of the method is 0.05 µg/L for each analyte.

Chromatography conditions

Chromatography Conditions for Pinoxaden, SYN546108, NOA407854 and NOA447204

Column	:	ACE C18 50 x 2.1 mm 3 mm
Column Oven Temperature	:	40 ºC
Injection volume	:	10 mL
Stop Time	:	9.0 mins
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: Methanol Solvent 2: 0.1% HCOOH in ultra-pure water

Mobile Phase Composition

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0	10	90	0.3
4.0	90	10	0.3
5.0	90	10	0.3
5.1	10	90	0.3
9.0	10	90	0.3

Divert Valve Switching Times

Time (mins)	Position
0	Waste
4.0	Mass spectrometer
8.0	Waste

Under these conditions the retention times are:

Analyte	**Approximate Retention Time (min)**
Pinoxaden	6.5
NOA407854	5.7
SYN546108	4.7
NOA447204	5.8

Mass Spectrometer Conditions for Pinoxaden, SYN546108, NOA407854 and NOA447204

Interface	:	TurboIonSpray
Polarity	:	Positive
Curtain gas (CUR)	:	Nitrogen set at 20 (arbitrary units)
Temperature (TEM)	:	450 °C
Ionspray voltage	:	4500 V
Collision gas setting (CAD)	:	Nitrogen set at 10
Gas 1 (GS1)	:	Air set at 35 (arbitrary units)
Gas 2 (GS2)	:	Air set at 35 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Conditions		**Pinoxaden primary transition**	**Pinoxaden confirmatory transition**	**NOA407854 primary transition**	**NOA407854 confirmatory transition**
Q1 *m/z*	:	401	401	317	317
Q3 *m/z*	:	317	57	115	91
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	72 V	72 V	101 V	101 V
Entrance potential (EP)	:	10 V	10 V	10 V	10 V
Collision energy (CE)	:	33 V	49 V	87 V	81 V
Collision cell exit potential (CXP)	:	22 V	8 V	8 V	8 V

MRM Conditions		**SYN546108 primary transition**	**SYN546108 confirmatory transition**	**NOA447204 primary transition**	**NOA447204 confirmatory transition**
Q1 *m/z*	:	343	343	333	333
Q3 *m/z*	:	243	115	149	121
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	101 V	101 V	56 V	56 V
Entrance potential (EP)	:	10 V	10 V	10 V	10 V
Collision energy (CE)	:	35 V	89 V	19 V	35 V
Collision cell exit potential (CXP)	:	16 V	10 V	8 V	8 V

Chromatography Conditions for SYN504574, SYN546105, SYN546106 and SYN546107

Column	:	ACE C18 50 x 2.1 mm 3 mm
Column Oven Temperature	:	40 ºC
Injection volume	:	80 mL
Stop Time	:	10.0 mins
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: methanol Solvent 2: 0.1% HCOOH in ultra-pure water

Mobile Phase Composition

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0	10	90	0.3
2.0	10	90	0.3
5.0	90	10	0.3
5.1	90	10	0.3
5.5	10	90	0.3
10	10	90	0.3

Divert Valve Switching Times

Time (mins)	Position
0	Waste
0.5	Mass spectrometer
8.0	Waste

Under these conditions the retention times are:

Analyte	**Approximate Retention Time (min)**
SYN504574	6.5
SYN546105	6.7
SYN546106	6.7
SYN546107	6.3

Mass Spectrometer Conditions for SYN504574, SYN546105, SYN546106 and SYN546107

Interface	:	TurboIonSpray
Polarity	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 20 (arbitrary units)
Temperature (TEM)	:	550 °C
Ionspray voltage	:	-4500 V
Collision gas setting (CAD)	:	Nitrogen set at 10
Gas 1 (GS1)	:	Air set at 35 (arbitrary units)
Gas 2 (GS2)	:	Air set at 35 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Conditions		**SYN504574 primary transition**	**SYN504574 confirmatory transition**	**SYN546105 primary transition**	**SYN546105 confirmatory transition**
Q1 *m/z*	:	361	361	359	359
Q3 *m/z*	:	300	305	159	144
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-80 V	-80 V	-90 V	-90 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V	-10 V
Collision energy (CE)	:	-38 V	-22 V	-48 V	-66 V
Collision cell exit potential (CXP)	:	-21 V	-21 V	-9 V	-9 V

MRM Conditions		SYN546106 primary transition	SYN546106 confirmatory transition	SYN546107 primary transition	SYN546107 confirmatory transition
Q1 *m/z*	:	361	361	375	375
Q3 *m/z*	:	173	217	271	241
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-65 V	-65 V	-80 V	-80 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V	-10 V
Collision energy (CE)	:	-38 V	-26 V	-28 V	-34 V
Collision cell exit potential (CXP)	:	-11 V	-15 V	-19 V	-17 V

Sample Preparation

a) If water samples are received deep frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity prior to analysis. Any particulates may be removed by centrifugation at a speed which visibly separates the particulates from the water.
b) Transfer water samples (20 mL) into suitable screw capped plastic tubes (40 mL size).

Recovery Findings

Summaries of the results for pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 are presented in the tables below.

Table A 24: Recovery and precision results from validation of GRM017.06A for pinoxaden in water: primary transition *m*/z 401 → 317

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 401 → 317)					
Surface Water	0.05	71,72, 68, 72, 73	71	2.9	68-73
	0.5	72, 65, 72, 70,72	70	4.2	65-72
	Overall		71	3.5	65-73
Groundwater	0.05	79, 78, 85, 75, 79	79	4.4	75-85
	0.5	82, 75, 70, 70, 77	75	6.8	70-82
	Overall		77	6.1	70-85

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 25: Recovery and precision results from validation of GRM017.06A for pinoxaden in water: confirmatory transition *m*/z 401 → 57

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 401 → 57)					

Surface Water	0.05	71, 72, 68, 74, 75	72	3.5	68-75
	0.5	71, 65, 71, 72, 73	70	4.2	65-73
	Overall		71	3.8	65-75
Groundwater	0.05	78, 79, 83, 77, 75	78	3.7	75-83
	0.5	80, 73, 70, 70, 78	74	6.6	70-80
	Overall		76	5.7	70-83

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 26: Recovery and precision results from validation of GRM017.06A for NOA407854 in water: primary transition m/z 317 → 115

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 317 → 115)					
Surface Water	0.05	92, 95, 92, 97, 99	95	3.3	92-99
	0.5	93, 92, 91, 90, 91	92	1.4	90-93
	Overall		93	3.1	90-99
Groundwater	0.05	83, 84, 84, 85, 84	84	1.1	83-85
	0.5	85, 85, 86, 83, 95	87	5.5	83-95
	Overall		85	4.2	83-95

Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 27: Recovery and precision results from validation of GRM017.06A for NOA407854 in water: confirmatory transition m/z 317 → 91

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 317 → 91)					
Surface Water	0.05	92, 94, 98, 98, 95	95	2.8	92-98
	0.5	92, 89, 90, 92, 90	90	1.2	89-92
	Overall		93	3.4	89-98
Groundwater	0.05	82, 82, 85, 80, 84	82	2.7	80-85
	0.5	87, 84, 86, 85, 98	88	6.7	84-98
	Overall		85	6.0	80-98

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values.

Table A 28: Recovery and precision results from validation of GRM017.06A for NOA447204 in water: primary transition *m*/z 333 → 149

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 333→ 149)					
Surface Water	0.05	82, 89, 97, 97, 97	92	7.3	82-97
	0.5	91, 89, 88, 89, 91	90	1.4	88-91
	Overall		91	5.2	82-97
Groundwater	0.05	80, 90, 89, 83, 105	89	10.5	83-105
	0.5	89, 86, 89, 86, 87	87	1.6	86-89
	Overall		88	7.3	83-105

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 29: Recovery and precision results from validation of GRM017.06A for NOA447204 in water: confirmatory transition *m*/z 333 → 121

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 333→ 121)					
Surface Water	0.05	98, 90, 102, 100, 99	98	5.0	90-102
	0.5	89, 93, 92, 89, 89	91	1.9	89-93
	Overall		94	5.5	89-102
Groundwater	0.05	88, 83, 92, 92, 103	91	8.1	83-103
	0.5	92, 87, 89, 90, 90	90	2.3	87-92
	Overall		91	5.8	83-103

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 30: Recovery and precision results from validation of GRM017.06A for SYN504574 in water: primary transition *m*/z 361 → 300

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 361→ 300)					
Surface Water	0.05	90, 87, 87, 89, 88	88	1.7	87-90
	0.5	87, 85, 88, 87, 89	87	1.4	85-89
	Overall		88	1.6	85-90
Groundwater	0.05	105, 96, 102, 104, 94	100	4.7	94-105
	0.5	92, 92, 91, 90, 92	91	1.2	90-92
	Overall		96	5.9	90-105

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 31: Recovery and precision results from validation of GRM017.06A for SYN504574 in water: confirmatory transition *m*/z 361 → 305

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 361→ 305)					
Surface Water	0.05	94, 91, 93, 89, 88	91	3.1	88-94
	0.5	87, 86, 89, 91, 87	88	1.9	86-91
	Overall		90	3.1	86-94
Groundwater	0.05	114, 106, 101, 108, 101	106	5.2	101-114
	0.5	95, 95, 94, 90, 95	94	2.2	90-95
	Overall		100	7.5	90-114

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 32: Recovery and precision results from validation of GRM017.06A for SYN546105 in water: primary transition *m*/z 359 → 159

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 359→ 159)					
Surface Water	0.05	86, 104, 91, 90, 89	92	7.9	86-104
	0.5	82, 82, 84, 83, 83	83	0.7	82-84
	Overall		87	7.9	82-104

Groundwater	0.05	88, 104, 97, 77, 91	91	11.0	77-104
	0.5	85, 88, 84, 86, 88	86	2.1	84-88
	Overall		89	8.3	77-104

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 33: Recovery and precision results from validation of GRM017.06A for SYN546105 in water: confirmatory transition *m*/z 359 → 144

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 359→ 144)					
Surface Water	0.05	112, 101, 94, 107, 97	102	7.3	94-112
	0.5	83, 82, 86, 84, 86	84	2.0	82-86
	Overall		93	11.7	82-112
Groundwater	0.05	72, 78, 105, 83, 109	89	18.2	83-109
	0.5	90, 89, 85, 88, 91	89	2.4	85-91
	Overall		89	12.3	83-109

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 34: Recovery and precision results from validation of GRM017.06A for SYN546106 in water: primary transition *m*/z 361 → 173

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 361→ 173)					
Surface Water	0.05	98, 79, 89, 88, 87	88	7.8	79-98
	0.5	93, 87, 88, 89, 88	89	2.6	87-93
	Overall		88	5.5	79-98
Groundwater	0.05	99, 95, 81, 97, 106	96	9.8	81-106
	0.5	102, 97, 99, 102, 105	101	3.3	97-105
	Overall		98	7.4	81-106

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 35: Recovery and precision results from validation of GRM017.06A for SYN546106 in water: confirmatory transition *m*/z 361 → 217

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 361→ 217)					
Surface Water	0.05	91, 95, 87, 87, 99	92	5.7	87-99
	0.5	86, 89, 89, 94, 92	90	3.2	86-94
	Overall		91	4.6	86-99
Groundwater	0.05	107, 102, 110, 91, 104	103	7.2	91-110
	0.5	104, 102, 98, 102, 99	101	2.3	98-104
	Overall		102	5.2	91-110

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 36: Recovery and precision results from validation of GRM017.06A for SYN546107 in water: primary transition *m*/z 375 → 271

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 375→ 271)					
Surface Water	0.05	97, 97, 111, 96, 98	100	6.6	96-111
	0.5	89, 88, 91, 90, 85	88	2.5	85-91
	Overall		94	8.1	85-111
Groundwater	0.05	106, 98, 103, 101, 103	102	3.0	98-106
	0.5	93, 90, 89, 90, 90	91	1.8	89-93
	Overall		96	6.8	89-106

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 37: Recovery and precision results from validation of GRM017.06A for SYN546107 in water: confirmatory transition *m*/z 375 → 241

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 375→ 241)					
Surface Water	0.05	99, 95, 93, 98, 103	98	4.2	93-103
	0.5	87, 86, 86, 90, 88	87	2.0	86-90
	Overall		92	6.7	86-103
Groundwater	0.05	106, 92, 87, 94, 94	95	7.4	87-106
	0.5	90, 87, 87, 90, 92	89	2.8	87-92
	Overall		92	6.2	87-106

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 38: Recovery and precision results from validation of GRM017.06A for SYN546108 in water: primary transition *m*/z 343 → 243

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Primary Transition *m/z* 343→ 243)					
Surface Water	0.05	93, 96, 93,93 ,92	93	1.6	92-96
	0.5	97, 96, 96, 94, 93	95	1.8	93-97
	Overall		94	1.9	92-97
Groundwater	0.05	88, 89, 90, 89, 97	91	3.9	88-97
	0.5	97, 90, 92, 97, 95	94	3.3	90-97
	Overall		92	4.0	88-97

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Table A 39: Recovery and precision results from validation of GRM017.06A for SYN546108 in water: confirmatory transition *m*/z 343 → 115

Matrix	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range (%)
(Confirmatory Transition *m/z* 343→ 115)					
Surface Water	0.05	101, 106, 104, 98, 104	103	3.0	98-106
	0.5	96, 100, 97, 97, 99	98	1.6	96-100
	Overall		100	3.3	96-106

Groundwater	0.05	94, 96, 89, 90, 99	93	4.3	89-99
	0.5	92, 92, 95, 89, 92	92	2.1	89-95
	Overall		93	3.3	89-95

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ
% Mean and % RSD calculated using un-rounded values

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the water matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using both non-matrix calibration standard solutions and matrix-matched standard solutions (from 0.3 to 15.0 ng/mL). Standards at seven different concentrations (0.3, 0.5, 1, 5, 10, 12.5 and 15 µg/L) were injected once per analytical run and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9980 to 1.0000 were obtained for a pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108.

LC-MS/MS Calibration for Non-Matrix Matched Pinoxaden Standards in 0.2% Formic Acid in Water/Acetonitrile (90/10 v/v)

Primary transition: $y = 910886.3x-139032.1$, $r^2 = 0.9993$
Confirmatory Transition: $y = 387453.3x-11131.6$, $r^2 = 0.9996$

LC-MS/MS Calibration for Non-Matrix Matched NOA407854 Standards in 0.2% Formic Acid in Water/Acetonitrile (90/10 v/v)

Primary transition: $y= 49975.3x-1470.7$, $r^2 = 0.9998$
Confirmatory Transition: $y= 87365.2x- 580.4$, $r^2 = 0.9995$

LC-MS/MS Calibration for Non-Matrix Matched NOA447204 Standards 0.2% Formic Acid in Water/Acetonitrile (90/10 v/v)

Primary transition: $y= 24669.5x-1577.3$, $r^2 = 0.9997$
Confirmatory Transition: $y= 11445.8x-659.7$, $r^2 = 0.9996$

LC-MS/MS Calibration for Non-Matrix Matched SYN546108 Standards 0.2% Formic Acid in Water/Acetonitrile (90/10 v/v)

Primary transition: $y= 114227.1x-5335.6$, $r^2 = 0.9997$
Confirmatory Transition: $y= 25153.3x-2620.1$, $r^2 = 0.9992$

LC-MS/MS Calibration for Groundwater Matrix Matched SYN504574 Standards

Primary transition: $y= 24709.7x - 2379.1$, $r^2 = 0.9996$
Confirmatory Transition: $y=12808.4x – 564.0$, $r^2 = 0.9997$

LC-MS/MS Calibration for Groundwater Matrix Matched SYN546105 Standards

Primary transition: $y=8044.8x - 797.6$, $r^2 = 0.9987$
Confirmatory Transition: $y=2684.6x – 512.2$, $r^2 = 0.9983$

LC-MS/MS Calibration for Groundwater Matrix Matched SYN546106 Standards

Primary transition: $y=5460.2x – 2250.5$, $r^2 = 0.9995$
Confirmatory Transition: $y=17561.8x – 3457.4$, $r^2 = 0.9993$

LC-MS/MS Calibration for Groundwater Matrix Matched SYN546107 Standards

Primary transition: $y=16426.2x – 1484.5$, $r^2 = 0.9983$

Confirmatory Transition: y=8225.9x – 307.3, r^2 = 0.9997

LC-MS/MS Calibration for Surface Water Matrix Matched SYN504574 Standards
Primary transition: y=41837.9x – 3663.9, r^2 = 0.9997
Confirmatory Transition: y=25082.1x – 229.2, r^2 = 0.9997

LC-MS/MS Calibration for Surface Water Matrix Matched SYN546105 Standards
Primary transition: y=13133.0x – 141.0, r^2 = 0.9984
Confirmatory Transition: y=4567.4x – 375.8, r^2 = 0.9992

LC-MS/MS Calibration for Surface Water Matrix Matched SYN546106 Standards
Primary transition: y=12521.6x – 5120.2, r^2 = 0.9989
Confirmatory Transition: y=39004.4x – 4983.0, r^2 = 1.0000

LC-MS/MS Calibration for Surface Water Matrix Matched SYN546107 Standards
Primary transition: y=32270.0x – 2929.9, r^2 = 0.9994
Confirmatory Transition: y=16549.4x – 1375.2, r^2 = 0.9996

Accuracy
Fortified samples were analysed in quintuplicate at the limit of quantification (LOQ) of 0.05 µg/L and at ten times the LOQ (0.5 µg/L). Acceptable mean accuracy values of between 70% and 120% were found for both transitions on matrices tested and therefore according to EU guidance (see guidance section of this summary), demonstrate the method has satisfactory accuracy.

Precision (Repeatability)
The relative standard deviations (RSDs) of pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 values at each fortification level and overall for the water samples tested during method validation were <20% and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.

Limit of Quantification
The LOQ for pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 residues in water using method GRM017.06A was established at 0.05 µg/L. No interfering peaks around the retention time of pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106 and SYN546107, SYN546108 were found in any of the control samples at levels above 30% of the LOQ.

Matrix Extract
No significant matrix effects (suppression or enhancement) were observed for pinoxaden, NOA407854, NOA447204 and SYN546108 in either of the water types used during the method validation. Significant matrix effects (suppression) was observed for SYN504574, SYN546105, SYN546106 and SYN546107 in the presence of groundwater and surface water.

Stability of Final Extracts
The stability of the sample extracts fortified with a pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 mixed standard was checked after a storage period of 7 days at 2-8 °C against freshly prepared calibration standards. The results indicate that pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 residues in stored fortified groundwater samples were stable for at least 7 days when stored at 2-8 °C and that NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 residues in stored fortified surface water samples were stable for at least 7 days when stored at 2-8 °C. Residues of pinoxaden were not stable in fortified surface water after 7 days when stored in vials at between 2-8 °C.

Stability of Standard Solutions
The stability of the stored working standard solutions were checked against freshly prepared calibration standards after a storage period of 37 days at 2-8 °C for SYN546107 and SYN546108 and after a storage period of 38 days at 2-8 °C for pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105 and SYN546106. The mean response values for stored and fresh solutions were within 10% of each other and the results demonstrated that pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 residues were stable in the standard solutions.

Conclusion
Analytical method GRM017.06A has been demonstrated to be a reliable and accurate procedure for the determination of pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107. and SYN546108 in water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents. No ILV for drinking water is required according to the guidance document on the interpretation of the transitional measures for the data requirements for chemical active substances and plant protection products according to Regulation (EU) No 283/2013 and Regu-lation (EU) No 284/2013 (SANTE/11509 /2013– rev. 5.2, 9 October 2015).

(Langridge, 2015)

Comments of zRMS:	Analytical method GRM017.06B has been acceptably validated for the determination of pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in groundwater and surface water with LOQ of 0.025 µg/L. The method is acceptable.

Reference:	KCP 5.2/04
Report	Langridge G. , Crook S. (2017). Pinoxaden - Residue Method GRM017.06B for the Determination of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 in Water by Direct Injection LC-MS/MS Analysis. Syngenta Analytical Method (Report Number) GRM017.06B. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Syngenta File No. VV-132772 (NOA407855_10407).
Reference:	KCP 5.2/05
Report	Langridge G. (2017). Pinoxaden - Validation of Draft Residue Method GRM017.06A for the Determination of Pinoxaden and Its Metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in Water. CEMAS Report Number CEMR-7546. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Syngenta File No. VV-466642 (NOA407855_10406)
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000) Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012
Deviations:	No

GLP:	Validation: Yes; Method: No
Acceptability:	Yes

Principle of the Method
For SYN546105, SYN546106, SYN546107 and SYN504574, water samples are taken through a solid phase extraction (SPE) clean-up procedure, using Waters Oasis™ HLB cartridges. The analytes are eluted from the SPE cartridge with acidified acetonitrile and then concentrated for analysis by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) in negative ionisation mode.

For Pinoxaden, NOA407854, NOA447204 and SYN546108, water samples are analysed after acidification by direct injection LC-MS/MS in positive ionisation mode.

The limit of quantification of the method is 0.025 µg/L (0.025 ppb)

Recovery Findings
Summaries of the results for Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 are presented in Tables A 44 to A 59.

Table A 30: Re Recovery results from validation of GRM017.06B for Pinoxaden in surface water and groundwater: quantitation transition m/z 401 → 317

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	99, 93, 98, 101, 98	5	98	3.0	93-101
	0.25	97, 92, 101, 94, 94	5	96	3.7	92-101
	Overall	-	10	97	3.4	92-101
Surface water	0.025*	93, 103, 90, 97, 99	5	96	5.3	90-103
	0.25	99, 99, 95, 94, 97	5	97	2.4	94-99
	Overall	-	10	97	3.8	90-103

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding

Table A 31: Recovery results from validation of GRM017.06B for Pinoxaden in surface water and groundwater: confirmatory transition m/z 401 → 57

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	108, 109, 105, 103, 111	5	107	3.0	103-111
	0.25	97, 92, 101, 94, 95	5	96	3.6	92-101
	Overall	-	10	102	6.7	92-111
Surface water	0.025*	96, 105, 102, 99, 106	5	102	4.1	96-106
	0.25	102, 99, 107, 95, 98	5	100	4.5	95-107
	Overall	-	10	101	4.1	95-107

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding

Table A 32: Recovery results from validation of GRM017.06B for NOA447204 in water: quantitation transition m/z 333 → 149

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	107, 91, 89, 90, 101	5	96	8.4	89-107
	0.25	101, 100, 98, 99, 99	5	99	1.1	98-101
	Overall	-	10	98	5.9	89-107
Surface water	0.025*	104, 104, 90, 97, 99	5	99	5.9	90-104
	0.25	99, 95, 97, 97, 95	5	97	1.7	95-99

	Overall	-	10	98	4.3	90-104

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 33: Recovery results from validation of GRM017.06B for NOA447204 in water: confirmatory transition m/z 333 → 121

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	87, 84, 104, 90, 99	5	93	9.1	84-104
	0.25	97, 94, 100, 103, 93	5	97	4.3	93-103
	Overall	-	10	95	7.1	84-104
Surface water	0.025*	87, 110, 112, 72, 106	5	97	17.8	72-112
	0.25	100, 95, 98, 102, 102	5	99	3.0	95-102
	Overall	-	10	98	11.9	72-112

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 34: Recovery results from validation of GRM017.06B for SYN546108 in water: quantitation transition m/z 343 → 243

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	113, 109, 112, 110, 114	5	112	1.9	109-114
	0.25	109, 109, 111, 110, 108	5	109	1.0	108-111
	Overall	-	10	111	1.8	108-114
Surface water	0.025*	98, 108, 102, 98, 101	5	101	4.0	98-108
	0.25	107, 103, 105, 106, 105	5	105	1.4	103-107
	Overall	-	10	103	3.4	98-108

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 35: Recovery results from validation of GRM017.06B for SYN546108 in water: confirmatory transition m/z 343 → 115

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	104, 112, 107, 106, 113	5	108	3.6	104-113
	0.25	110, 111, 111, 109, 105	5	109	2.3	105-111
	Overall	-	10	109	2.9	104-113
Surface water	0.025*	89, 112, 107, 83, 96	5	97	12.4	83-112
	0.25	104, 103, 104, 100, 103	5	103	1.6	100-104
	Overall	-	10	100	8.6	83-112

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 36: Recovery results from validation of GRM017.06B for NOA407854 in water: quantitation transition m/z 317 → 115

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	102, 101, 102, 101, 106	5	102	2.0	101-106
	0.25	97, 98, 104, 102, 98	5	100	3.0	97-104
	Overall	-	10	101	2.8	97-106
Surface water	0.025*	104, 105, 95, 92, 102	5	100	5.8	92-105
	0.25	101, 100, 99, 100, 99	5	100	0.8	99-101
	Overall	-	10	100	3.9	92-105

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 37: Recovery results from validation of GRM017.06B for NOA407854 in water: confirmatory transition m/z 317 → 91

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	103, 106, 104, 102, 101	5	103	1.9	101-106
	0.25	101, 100, 101, 101, 98	5	100	1.3	98-101
	Overall	-	10	102	2.2	98-106
Surface water	0.025*	108, 105, 96, 100, 100	5	102	4.6	96-108
	0.25	98, 101, 100, 104, 100	5	101	2.2	98-104
	Overall	-	10	101	3.5	96-108

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 38: Recovery results from validation of GRM017.06B for SYN504574 in water: quantitation transition m/z 361 → 300

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	85, 80, 82, 88, 82	5	83	3.8	80-88
	0.25	80, 83, 81, 82, 84	5	82	1.9	80-84
	Overall	-	10	83	3.0	80-88
Surface water	0.025*	69, 71, 75, 77, 77	5	74	4.9	69-77
	0.25	70, 73, 71, 72, 71	5	71	1.6	70-73
	Overall	-	10	73	3.9	69-77

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 39: Recovery results from validation of GRM017.06B for SYN504574 in water: confirmatory transition m/z 361 → 305

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	84, 87, 83, 87, 87	5	86	2.3	83-87
	0.25	79, 80, 80, 79, 83	5	80	2.0	79-83
	Overall	-	10	83	4.0	79-87
Surface water	0.025*	81, 80, 82, 80, 75	5	80	3.4	75-82
	0.25	75, 75, 80, 78, 76	5	77	2.8	75-80
	Overall	-	10	78	3.5	75-82

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 40: Recovery results from validation of GRM017.06B for SYN546107 in water: quantitation transition m/z 375→ 271

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	96, 92, 87, 97, 93	5	93	4.2	87-97
	0.25	75, 85, 76, 83, 87	5	81	6.7	75-87
	Overall	-	10	87	8.8	75-97
Surface water	0.025*	103, 106, 92, 89, 86	5	95	9.3	86-106
	0.25	73, 73, 77, 73, 75	5	74	2.4	73-77
	Overall	-	10	85	14.9	73-106

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 41: Recovery results from validation of GRM017.06B for SYN546107 in water: confirmatory transition m/z 375→ 241

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	103, 108, 103, 105, 106	5	105	2.0	103-108
	0.25	77, 83, 76, 86, 90	5	82	7.2	76-90

	Overall	-	10	94	13.5	76-108
Surface water	0.025*	74, 90, 70, 94, 76	5	81	13.1	70-94
	0.25	82, 80, 78, 81, 89	5	82	5.1	78-89
	Overall	-	10	81	9.3	70-94

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 42: Recovery results from validation of GRM017.06B for SYN546105 in water: quantitation transition *m*/z 359 → 159

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	89, 79, 83, 93, 84	5	86	6.4	79-93
	0.25	77, 83, 77, 78, 83	5	80	3.9	77-83
	Overall	-	10	83	6.4	77-93
Surface water	0.025*	72, 70, 82, 97, 75	5	79	13.8	70-97
	0.25	80, 88, 75, 80, 84	5	81	6.0	75-88
	Overall	-	10	80	10.0	70-97

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 43: Recovery results from validation of GRM017.06B for SYN546105 water: confirmatory transition *m*/z 359 → 144

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	89, 79, 83, 82, 81	5	83	4.6	79-89
	0.25	79, 82, 77, 80, 82	5	80	2.7	77-82
	Overall	-	10	81	4.0	77-89
Surface water	0.025*	74, 76, 80, 75, 71	5	75	4.3	71-80
	0.25	70, 79, 74, 75, 73	5	74	4.4	70-79
	Overall	-	10	75	4.2	70-80

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 44: Recovery results from validation of GRM017.06B for SYN546106 in water: quantitation transition *m*/z 361 → 217

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	87, 81, 83, 91, 83	5	85	4.7	81-91
	0.25	82, 83, 83, 82, 88	5	84	3.0	82-88
	Overall	-	10	84	3.8	81-91
Surface water	0.025*	72, 65, 82, 75, 74	5	74	8.3	65-82
	0.25	80, 88, 78, 81, 85	5	82	4.9	78-88
	Overall	-	10	78	8.6	65-88

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Table A 45: Recovery results from validation of GRM017.06B for SYN546106 water: confirmatory transition *m*/z 361 → 173

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Groundwater	0.025*	80, 68, 89, 96, 95	5	86	13.7	68-96
	0.25	82, 88, 82, 89, 88	5	86	4.1	82-89
	Overall	-	10	86	9.5	68-96
Surface water	0.025*	80, 83, 104, 93, 78	5	88	12.4	78-104
	0.25	86, 92, 83, 77, 75	5	83	8.3	75-92
	Overall	-	10	85	10.5	75-104

*0.025 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ.
Tables were calculated using Microsoft Excel therefore minor discrepancies may be present due to rounding.

Accuracy (Analytical Recovery)
A reagent blank sample was analysed, control samples were analysed in duplicate and fortified samples were analysed in quintuplet at the 0.025 µg/L and in quintuplet at a higher level (0.25 µg/L).
Acceptable mean recovery of the calculated concentration of between 70% and 120% of the nominal fortified concentration with a mean precision lower than 20% were found using both primary and confirmatory transitions and therefore according to EU guidance (see guidance section of this summary) demonstrate the method has satisfactory accuracy (analytical recovery).

Repeatability and Specificity
The repeatability and specificity of the method have been demonstrated and draft analytical method GRM017.06B has been successfully validated for the determination of residues of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in groundwater and surface water at the LOQ of 0.025 µg/L.

Matrix Effects
Significant suppression or enhancement of detector response was seen for some of the analytes in the presence of the water types used in this validation. It is recommended that matrix-matched calibration standards are used for analysis.

Linearity
The linearity of the LC-MS/MS detector response for Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 was tested at concentrations ranging from 0.0075 to 0.50 µg/L) using the conditions specified in the analytical method The lowest concentration injected was at 30% of the LOQ of the method, the upper margin was at least equivalent to the highest fortification level of the samples..

Limit of Quantification
The limit of quantification (LOQ) of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 residues using method GRM017.06B was established at 0.025 µg/L in groundwater and surface water. No interfering peaks around the retention time of the analytes were found in any of the control samples or reagent blank samples at levels above 30% of the LOQ.

Limit of Detection
For both primary and confirmatory transitions of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in water the baseline noise of one control sample was measured. The baseline noise was multiplied by 3 and then compared to the intensity of the standard corresponding to the LOQ. The LOD for Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in groundwater and surface water was estimated.

Stability of Final Extracts
Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 are stable in fortified groundwater samples for at least 6 days when stored in vials at between 2-8 °C. Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 are stable in fortified surface water samples for at least 6 days when stored in vials at between 2-8 °C. Pinoxaden is not stable in fortified surface water after 6 days when stored in vials at between 2-8 °C.

Stability of Standard Solutions
The stability of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 standard solutions in acetonitrile/methanol (90/10, v/v) was previously assessed in a separate GLP Study (CEMS-7135-INT1) by comparing dilutions of freshly prepared standard solutions with dilutions of stored standard solutions. Pinoxaden, NOA407854, NOA447204,

SYN504574, SYN546105, SYN546107, SYN546108 and SYN546106 were found to be stable up to 104 days stored between 2 - 8°C.

Conclusion
Analytical method GRM017.06B has been demonstrated to be a reliable and accurate procedure for the determination of Pinoxaden, NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107 and SYN546108 in groundwater and surface water to a limit of quantification of 0.025 µg/L, using commercially available laboratory equipment and reagents.

Comments of zRMS:	The ILV of the method GRM017.06B is acceptable.

Reference:	KCP 5.2/~~04~~ 06
Report	Watson G (2017). Pinoxaden - Independent Laboratory Validation (ILV) of analytical method GRM017.06B for the determination of Pinoxaden (NOA407855) and metabolites NOA407854, NOA447204, SYN504574, SYN546105, SYN546106, SYN546107, SYN546108 in Water. RES-00108 ResChem Analytical Limited, Unit 27 Derwent Business Centre, Clarke Street, Derby, Unit-ed Kingdom. DE1 2BU Syngenta File No. VV-468411
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012.
Deviations:	No
GLP:	Validation: Yes
Acceptability:	Yes/No/Supplementary

Principle of the Method
In summary, surface water and groundwater samples were analysed directly by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) for pinoxaden, NOA407854, NOA447204 and SYN546108. The limit of quantification (LOQ) of the method is 0.025 µg/L for each analyte.
In summary, surface water and groundwater samples were taken through a solid phase extraction (SPE) clean-up procedure, using Waters Oasis™ HLB cartridges. The analytes were eluted from the SPE cartridge with acidified acetonitrile and then concentrated for analysis by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) for SYN546105, SYN546106, SYN546107 and SYN504574. The limit of quantification (LOQ) of the method is 0.025 µg/L for each analyte.

Recovery Findings
Summaries of the results for Pinoxaden (NOA407855), NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and SYN504574 are presented in
Table A 46 to Table A 61.

Table A 46: Accuracy and precision results from independent laboratory validation of GRM017.06B for pinoxaden in water primary transition m/z 401 → 317

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	99, 108, 105, 111, 109	5	107	4.4	99 - 111
	0.25	105, 102, 108, 104 108	5	105	2.5	102 - 108
	Overall		10	106	3.5	99 - 111
Groundwater	0.025*****	103, 129, 117, 108, 104	5	112	9.9	103 - 129
	0.25	93, 92, 96, 100, 96	5	95	3.0	92 - 100
	Overall		10	104	11.2	92 - 129

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 47: Accuracy and precision results from independent laboratory validation of GRM017.06B for pinoxaden in water confirmatory transition m/z 401 → 57

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*	105, 104, 104, 110, 110	5	106	2.8	104 - 110
	0.25	100, 99, 104, 103, 104	5	102	2.2	99 - 104
	Overall		10	104	3.3	99 - 110
Groundwater	0.025*	104, 127, 110, 119, 109	5	114	7.7	104 - 127
	0.25	93, 92, 95, 98, 96	5	95	2.2	92 - 98
	Overall		10	104	11.2	92 - 127

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 48: Accuracy and precision results from independent laboratory validation of GRM017.06B for NOA407854 in water primary transition m/z 317 → 115

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean Accuracy (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	104, 104, 111, 109, 106	5	107	2.8	104-111
	0.25	107, 105, 106, 108, 108	5	107	1.2	105-108
	Overall		10	107	2.0	104-111
Groundwater	0.025*****	101, 100, 106, 109, 102	5	103	3.8	100-109
	0.25	106, 111, 105, 104, 104	5	106	2.8	104-111
	Overall		10	105	3.4	100-111

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 49: Accuracy and precision results from independent laboratory validation of GRM017.06B for NOA407854 in water confirmatory transition m/z 317 → 91

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*	105, 102, 106, 109, 106	5	105	2.5	102-109
	0.25	103, 104, 109, 108, 106	5	106	2.4	103-109
	Overall		10	106	2.3	102-109
Groundwater	0.025*	105, 107, 108, 107, 104	5	106	1.6	104-108
	0.25	102, 111, 106, 103, 100	5	104	4.0	100-111
	Overall		10	105	3.0	100-111

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 50: Accuracy and precision results from independent laboratory validation of GRM017.06B for NOA447204 in water primary transition m/z 333 → 149

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	105, 96, 104, 102, 106	5	103	3.7	96-106
	0.25	105, 106, 105, 107, 108	5	106	1.2	105-108
	Overall		10	104	3.2	96-108
Groundwater	0.025*****	103, 106, 102, 108, 103	5	104	2.3	102-108
	0.25	104, 110, 106, 105, 105	5	106	2.1	104-110
	Overall		10	105	2.2	102-110

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 51: Accuracy and precision results from independent laboratory validation of GRM017.06B for NOA447204 in water confirmatory transition m/z 333 → 121

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	106, 98, 99, 98, 104	5	101	3.8	98-106
	0.25	103, 107, 108, 106, 107	5	106	1.8	98-108
	Overall		10	103	3.8	92-111
Groundwater	0.025*****	100, 107, 106, 100, 105	5	104	3.1	100-107
	0.25	105, 111, 104, 103, 105	5	106	3.0	103-111
	Overall		10	105	3.0	100-111

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 52: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN504574 in water primary transition m/z 361 → 300

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	97, 96, 98, 98, 95	5	97	1.3	95-98
	0.25	100, 100, 101, 99, 102	5	101	1.3	99-102
	Overall		10	99	2.4	95-102
Groundwater	0.025*****	95, 97, 96, 94, 96	5	96	1.1	94-97
	0.25	98, 96, 96, 98, 96	5	97	1.3	96-98
	Overall		10	96	1.3	94-98

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 53: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN504574 in water confirmatory transition m/z 361 → 305

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	99, 97, 104, 103, 98	5	100	3.1	97-104
	0.25	102, 98, 100, 94, 100	5	99	3.0	94-102
	Overall		10	99	3.0	94-104
Groundwater	0.025*****	100, 96, 95, 91, 96	5	96	3.2	91-100
	0.25	97, 98, 89, 95, 96	5	95	3.5	89-98
	Overall		10	95	3.2	89-100

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 54: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546105 in water primary transition m/z 359 → 159

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	97, 84, 82, 64, 70	5	80	16.3	64-97
	0.25	98, 96, 97, 92, 98	5	96	2.5	92-98
	Overall		10	88	14.0	64-98
Groundwater	0.025*****	95, 87, 84, 86, 85	5	87	4.9	84-95
	0.25	94, 90, 88, 92, 94	5	91	2.9	88-94
	Overall		10	89	4.5	84-95

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 55: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546105 in water confirmatory transition m/z 359 → 144

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	97, 88, 91, 104, 88	5	94	7.4	88-104
	0.25	93, 96, 101, 95, 96	5	96	3.0	93-101
	Overall		10	95	5.4	88-104
Groundwater	0.025*****	85, 85, 84, 87, 85	5	85	1.4	84-87
	0.25	92, 89, 91, 91, 94	5	91	2.0	89-94
	Overall		10	88	4.0	84-94

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 56: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546106 in water primary transition m/z 361 → 217

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	97, 78, 88, 94, 86	5	88	8.5	78-97
	0.25	94, 95, 97, 97, 98	5	96	1.8	94-98
	Overall		10	92	7.1	78-98
Groundwater	0.025*****	98, 93, 107, 94, 96	5	97	5.7	93-107
	0.25	98, 95, 98, 93, 103	5	97	3.8	93-103
	Overall		10	97	4.6	93-107

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 57: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546106 in water confirmatory transition m/z 361 → 173

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	120, 127, 103, 111, 111	5	114	8.0	103-127
	0.25	94, 94, 93, 96, 97	5	95	1.8	93-97
	Overall		10	104	11.6	93-127
Groundwater	0.025*****	108, 102, 92, 100, 102	5	101	5.8	92-108
	0.25	101, 98, 102, 95, 106	5	100	4.3	95-106
	Overall		10	101	4.8	92-108

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 58: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546107 in water primary transition m/z 375 → 271

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	91, 87, 95, 96, 102	5	94	6.0	87-102
	0.25	99, 98, 102, 98, 106	5	101	3.1	98-106
	Overall		10	97	5.6	87-106
Groundwater	0.025*****	101, 99, 94, 93, 104	5	98	4.5	93-104
	0.25	99, 98, 94, 97, 100	5	97	2.5	94-100
	Overall		10	98	3.5	93-104

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 59: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546107 in water confirmatory transition m/z 375 → 241

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	104, 103, 112, 106, 109	5	107	3.7	103-112
	0.25	97, 99, 100, 100, 101	5	99	1.5	97-101
	Overall		10	103	4.8	97-112
Groundwater	0.025*****	111, 106, 100, 97, 88	5	100	9.0	88-111
	0.25	97, 97, 94, 99, 97	5	97	1.7	94-99
	Overall		10	99	6.5	88-111

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 60: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546108 in water primary transition m/z 343 → 243

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	102, 101, 102, 105, 105	5	103	2.0	101-105
	0.25	103, 105, 109, 108, 107	5	106	2.2	103-109
	Overall		10	105	2.6	101-109
Groundwater	0.025*****	103, 105, 104, 106, 101	5	104	1.8	101-106
	0.25	103, 108, 104, 108, 102	5	105	2.7	102-108
	Overall		10	104	2.2	101-108

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Table A 61: Accuracy and precision results from independent laboratory validation of GRM017.06B for SYN546108 in water confirmatory transition m/z 343 → 115

Matrix	Fortification Level (µg/L)*	Accuracy (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Accuracy Range (%)
Surface Water	0.025*****	105, 103, 102, 107, 105	5	104	1.7	102-107
	0.25	102, 104, 108, 108, 108	5	106	2.5	102-108
	Overall		10	105	2.2	102-108
Groundwater	0.025*****	105, 104, 101, 102, 104	5	103	1.5	101-105
	0.25	105, 111, 106, 103, 103	5	106	2.9	103-111
	Overall		10	104	2.6	101-111

*Limit of quantitation, defined by the lowest validated fortification level
Residues in duplicate control samples and reagent blanks were less than 30% of the LOQ

Deviations from test guidelines

None.

Specificity
LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the water matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity
For Pinoxaden, NOA407854, NOA447204 and SYN546108 the linearity of the LC-MS/MS detector was tested using matrix-matched standard solutions (from 0.0075 to 0.3 µg/L; equivalence concentrations of 0.0075 to 0.3 µg/L). Standards at six different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9963 to 0.9996 were obtained for Pinoxaden, NOA407854, NOA447204 and SYN546108.
For SYN546105, SYN546106, SYN546107 and SYN504574 the linearity of the LC-MS/MS detector was tested using matrix-matched standard solutions (from 0.15 to 6.0 µg/L; equivalence concentrations of 0.0075 to 0.3 µg/L). Standards at six different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9982 to 0.9999 were obtained for Pinoxaden, SYN546105, SYN546106, SYN546107 and SYN504574.

Accuracy
Fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.025 µg/L and at ten times the LOQ (0.25 µg/L). Acceptable mean accuracy values of between 70% and 120% were found for both transitions on matrices tested and therefore according to EU guidance (see guidance section of this summary) demonstrate the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of Pinoxaden, NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and SYN504574 accuracy values at each fortification level and overall for the water samples tested during method validation were <20% and therefore according to the EU guidance (see guidance section of this summary) demonstrate the method has satisfactory repeatability.

Limit of Quantification
The LOQ for Pinoxaden, NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and SYN504574 residues in water using method GRM017.06B was established at 0.025 µg/L. No interfering peaks around the retention time of Pinoxaden, NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and SYN504574 were found in any of the control samples at levels above 30% of the LOQ.

Matrix Extract
No significant matrix effects were observed for Pinoxaden, NOA407854, NOA447204 and SYN546108 in the matrices tested during the method validation. Significant matrix effects were observed for SYN546105, SYN546106, SYN546107 and SYN504574 and therefore, matrix matched linearity standards were used for quantification.

Stability of Final Extracts
The stability of the sample extracts fortified pinoxaden, NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and SYN504574 was checked after a storage period of at least 8 days at 2-8 ºC against freshly prepared calibration standards. The results proved that pinoxaden residues in the stored fortified water samples were not stable (the mean accuracy values were <70%) and that NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and

SYN504574 residues in the stored fortified water samples were stable (the mean accuracy values were between 70 – 120 % with an RSD ≤20%).

Stability of Standard Solutions

The stability of the stored working standard solutions of pinoxaden, NOA407854, NOA447204 and SYN546108 were checked after a storage period of 40 days at 2-8 ºC against freshly prepared calibration standards. The mean response values for stored and fresh solutions were within 10% of each other and the results demonstrated that pinoxaden, NOA407854, NOA447204 and SYN546108 residues were stable in the standard solutions.

The stability of the stored working standard solutions of SYN546105, SYN546106, SYN546107 and SYN504574 were checked after a storage period of 33 days at 2-8 ºC against freshly prepared calibration standards. The mean response values for stored and fresh solutions were within 10% of each other and the results demonstrated that SYN546105, SYN546106, SYN546107 and SYN504574 residues were stable in the standard solutions.

Conclusion

Analytical method GRM017.06B has been demonstrated to be a reliable and accurate procedure for the determination of Pinoxaden (NOA407855), NOA407854, NOA447204, SYN546108, SYN546105, SYN546106, SYN546107 and SYN504574 in water to a limit of quantification of 0.025 µg/L, using commercially available laboratory equipment and reagents.

(Watson, 2017)

Comments of zRMS:	Analytical method T001530-03 has been acceptably validated for the determination of residues of metabolites SYN505164 and SYN502836 in animal matrices (muscle, kidney, liver, fat, milk and eggs) by LC/LC-MS/MS with limit of quantification of 0.01 mg/kg for milk and 0.02 mg/kg for liver, kidney, muscle, fat and eggs. The method is acceptable.

Reference:	KCP 5.2/07
Report:	Pinoxaden (NOA407855) - Validation of Analytical Method T001530-03 for the Determination of Residues of Metabolites SYN505164 and SYN502836 in Animal Matrices by LC/LC-MS/MS, Homazava N. (2020), Report number 20190507 (TK0529647); VV-872393
Guideline(s):	OECD Guidance Document on Pesticide Residue Analytical methods ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007). European Commission Guidance Document SANCO/3029/99 rev. 4 (11 Jul 2000) for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. Complies with US EPA guideline Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174 (Aug 1996). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Principle of the method

The homogenized sample is extracted with 1N hydrochloric acid by boiling under reflux for two hours. After cooling to room temperature, an aliquot of the extract is cleaned-up by solid phase extraction and analyzed by LC/LC-MS/MS. The limit of quantification of the method is 0.01 mg/kg in milk and 0.02 mg/kg in liver, kidney, muscle, fat and eggs for SYN505164 and SYN502836.
The analytical residue Syngenta Method T001530-03 was validated for the determination of residues of SYN505164 and SYN502836 in animal matrices (muscle, kidney, liver, fat, milk and eggs).

Recovery Findings

Summaries of the results for SYN505164 and SYN502836 are presented in **Table A 62** to **Table A 74**.

Table A 63: Recovery results from validation of T001530-03 for SYN505164 in/on Pork Liver

Compound	Fortification Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 333/303 (Quantification)										
SYN505164	0.02*	64	81	87	83	64	5	76	15	64 - 87
	0.2	84	75	84	70	96	5	82	12	70 - 96
	Overall							79	13	64 - 96
Mass transition 333/117 (Confirmation)										
SYN505164	0.02*	66	84	82	82	63	5	75	14	63 - 84
	0.2	84	74	84	71	95	5	82	12	71 - 95
	Overall							78	13	63 - 95

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 64: Recovery results from validation of T001530-03 for SYN505164 in/on Pork Fat

Compound	Fortification Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 333/303 (Quantification)										
SYN505164	0.02*	95	107	105	108	89	5	101	8.1	89 - 108
	0.2	105	102	88	108	99	5	100	7.8	88 - 108
	Overall							101	7.5	88 - 108
Mass transition 333/117 (Confirmation)										
SYN505164	0.02*	101	109	109	106	95	5	104	5.8	95 - 109
	0.2	109	105	90	111	101	5	103	8.1	90 - 111
	Overall							103	6.6	90 - 111

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 65:: Recovery results from validation of T001530-03 for SYN505164 in/on Milk

Compound	Fortification Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 333/303 (Quantification)										
SYN505164	0.01*	89	104	106	110	93	5	100	9.2	89 - 110
	0.1	105	107	90	106	106	5	103	6.9	90 - 107
	Overall							102	7.7	89 - 110
Mass transition 333/117 (Confirmation)										

SYN505164	0.01*	95	110	108	113	94	5	104	8.6	94 - 113
	0.1	106	107	90	108	108	5	104	7.3	90 - 108
	Overall							104	7.5	90 - 113

* 0.01 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 66: Recovery results from validation of T001530-03 for SYN505164 in/on Eggs

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 333/303 (Quantification)										
SYN505164	0.02*	104	113	106	110	96	5	106	6.5	96 - 113
	0.2	105	104	91	96	81	5	95	10	81 - 105
	Overall							101	9.6	81 - 113
Mass transition 333/117 (Confirmation)										
SYN505164	0.02*	97	114	106	107	94	5	104	7.9	94 - 114
	0.2	104	101	91	96	81	5	94	9.5	81 - 104
	Overall							99	9.6	81 - 114

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 67: Recovery results from validation of T001530-03 for SYN505164 in/on Beef Muscle

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 333/303 (Quantification)										
SYN505164	0.02*	74	92	82	87	78	5	82	8.3	74 - 92
	0.2	92	88	89	89	102	5	92	6.4	88 - 102
	Overall							87	9.0	74 - 102
Mass transition 333/117 (Confirmation)										
SYN505164	0.02*	79	86	79	82	78	5	81	3.9	78 - 86
	0.2	92	89	88	90	103	5	92	6.8	88 - 103
	Overall							87	9.0	78 - 103

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 68: Recovery results from validation of T001530-03 for SYN505164 in/on Pork Kidney

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 333/303 (Quantification)										
SYN505164	0.02*	64	72	76	85	63	5	72	13	63 - 85
	0.2	87	76	76	76	76	5	78	6.2	76 - 87
	Overall							75	10	63 - 87
Mass transition 333/117 (Confirmation)										
SYN505164	0.02*	66	77	76	89	69	5	76	12	66 - 89
	0.2	90	77	76	75	76	5	79	7.5	75 - 90
	Overall							77	9.5	66 - 90

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 69: Recovery results from validation of T001530-03 for SYN502836 in/on Pork Liver

Compound	Forti-	Recoveries	No. of	Overall Recovery

	fication Level [mg/kg]	Single Values [%]					Analyses	Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 345/173 (Quantification)										
SYN502836	0.02*	78	88	93	83	74	5	83	9.2	74 - 93
	0.2	87	81	94	74	96	5	87	11	74 - 96
	Overall							85	9.7	74 - 96
Mass transition 345/217 (Confirmation)										
SYN502836	0.02*	77	86	99	83	76	5	84	11	76 - 99
	0.2	86	81	90	74	98	5	86	11	74 - 98
	Overall							85	10	74 - 99

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 70: Recovery results from validation of T001530-03 for SYN502836 in/on Pork Fat

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 345/173 (Quantification)										
SYN502836	0.02*	104	109	105	102	101	5	104	3.2	101 - 109
	0.2	110	109	103	109	97	5	105	5.5	97 - 110
	Overall							105	4.3	97 - 110
Mass transition 345/217 (Confirmation)										
SYN502836	0.02*	97	107	98	100	94	5	99	4.9	94 - 107
	0.2	104	100	98	104	93	5	100	4.8	93 - 104
	Overall							99	4.6	93 - 107

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 71: Recovery results from validation of T001530-03 for SYN502836 in/on Milk

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 345/173 (Quantification)										
SYN502836	0.01*	95	104	107	110	96	5	102	6.4	95 - 110
	0.1	106	108	100	108	110	5	106	3.7	100 - 110
	Overall							104	5.2	95 - 110
Mass transition 345/217 (Confirmation)										
SYN502836	0.01*	97	99	97	112	104	5	102	6.2	97 - 112
	0.1	102	102	100	105	107	5	103	2.9	100 - 107
	Overall							102	4.6	97 - 112

* 0.01 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 72: Recovery results from validation of T001530-03 for SYN502836 in/on Eggs

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]					No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 345/173 (Quantification)										
SYN502836	0.02*	105	112	108	110	104	5	108	3.2	104 - 112
	0.2	107	108	103	100	82	5	100	11	82 - 108

	Overall							104	8.3	82 - 112
Mass transition 345/217 (Confirmation)										
SYN502836	0.02*	106	110	104	109	105	5	107	2.5	104 - 110
	0.2	109	109	104	102	81	5	101	11	81 - 109
	Overall							104	8.2	81 - 110

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 73: Recovery results from validation of T001530-03 for SYN502836 in/on Beef Muscle

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]						No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 345/173 (Quantification)											
SYN502836	0.02*	86	91	84	87	83	5		86	3.5	83 - 91
	0.2	95	97	93	90	97	5		94	3.0	90 - 97
	Overall								90	5.7	83 - 97
Mass transition 345/217 (Confirmation)											
SYN502836	0.02*	80	90	87	92	92	5		88	5.4	80 - 92
	0.2	101	98	87	98	103	5		97	6.2	87 - 103
	Overall								93	7.5	80 - 103

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Table A 74: Recovery results from validation of T001530-03 for SYN502836 in/on Pork Kidney

Compound	Forti-fication Level [mg/kg]	Recoveries Single Values [%]						No. of Analyses	Overall Recovery Mean [%]	Rel. Std. Dev. [%]	Range [%]
Mass transition 345/173 (Quantification)											
SYN502836	0.02*	78	85	87	83	81	5		83	4.1	78 - 87
	0.2	93	85	86	91	85	5		88	4.4	85 - 93
	Overall								85	5.2	78 - 93
Mass transition 345/217 (Confirmation)											
SYN502836	0.02*	77	77	79	85	82	5		80	4.0	77 - 85
	0.2	91	85	83	90	83	5		86	4.7	83 - 91
	Overall								83	5.8	77 - 91

* 0.02 mg/kg = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30% of the LOQ.

Specificity

LC/LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidances (***SANCO/3029/99 rev. 4 (2000)***) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the animal matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the detector response was confirmed by injecting in duplicate seven matrix matched or solvent (depending on the matrix) calibration standards covering the working range of 0.1 ng/mL to 10 ng/mL. Linearity was tested for both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against the concentration for all calibration points. Straight lines with the coefficient of determination (R^2) of 0.9958 to 0.9996 were achieved for the primary transition of SYN502836 and between 0.9976 and 0.9995 for the confirmatory transition. For

SYN505164 the coefficient of determination (R^2) ranged between 0.9952 and 0.9997 for the primary transition and between 0.9948 and 0.9997 for the confirmatory transition.

Accuracy
Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg for milk and 0.02 mg/kg for liver, kidney, muscle, fat and eggs) and at in quintuplet at the 10x LOQ (0.10 mg/kg for milk and 0.2 mg/kg for liver, kidney, muscle, fat and eggs). Acceptable mean recoveries of between 70% and 110% were found for both primary and confirmatory mass transitions and for all matrices and therefore according to EU guidances (***SANCO 3029/99 rev.4 11/7/00***) demonstrate the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of SYN505164 and SYN502836 recoveries at each fortification level and overall during method validation were <20% and therefore according to the EU guidance (***SANCO 3029/99 rev.4 11/7/00***) demonstrate the method has satisfactory repeatability.

Limit of Quantification
The limit of quantification for SYN505164 and SYN502836 residues in animal matrices (muscle, kidney, liver, fat, milk and eggs) using analytical method T001530-03 was established at 0.01 mg/kg for milk and at 0.02 mg/kg for liver, kidney, muscle, fat and eggs. No interfering peaks around the retention time SYN505164 and SYN502836 were found in any of the control samples at levels above 30% of the limit of quantification.

Matrix Extract
Significant matrix effects (i.e. ≥20% suppression or enhancement) on the LC/LC-MS/MS detector response were observed in the following animal matrices: muscle, fat, milk and eggs. Therefore all sample extracts for these animal matrices were evaluated with multi-point calibrations based on matrix matched calibration standards.

For the kidney and liver matrices the matrix effects were found to be non-significant (i.e. <20% suppression or enhancement) and therefore solvent calibration standards were used for the quantification in kidney and liver matrices.

Stability of Final Extracts
The stability of the sample extracts fortified with SYN505164 and SYN502836 was checked after a storage period of 7-9 days in a refrigerator at 2-8°C against freshly prepared matrix matched calibration standards or solvent calibration standards (depending on the matrix). The overall mean recoveries in the stored fortified samples were within the acceptable range of 70-110% and within ±20% of the initial values for all matrices. The sample extracts can thus be considered as stable for 7 to 9 days.

Conclusion
The Analytical method has been demonstrated to be a reliable and accurate procedure for the determination of SYN505164 and SYN502836 in animal matrices (muscle, kidney, liver, fat, milk and eggs) to a limit of quantification of 0.01 mg/kg for milk and 0.02 mg/kg for liver, kidney, muscle, fat and eggs, using commercially available laboratory equipment and reagents.

Comments of zRMS:	Method QuEChERS has been acceptably validated for the determination of residues of pinoxaden (NOA407854) in bovine blood with a limit of quantification (LOQ) of 0.01 mg/L. The method complies with the data requirements given in SANTE/2020/12830. The method is acceptable.

Data point:	KCP 5.2/08
Reference:	TK0677616
Report:	Pinoxaden: Validation of Analytical Method QuEChERS for the Determination of Residues of NOA407854 in Body Fluid (Blood only) by LC-MS/MS, Bejan. I (2022), Report number S22-05825 VV-967942
Guideline(s):	EC 1107/2009 SANTE/2020/12830
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials

Test Material	Pinoxaden (NOA407854)
Lot/Batch #:	629148
Purity (%):	99 %
IUPAC name:	8-(2,6-diethyl-4-methyl-phenyl)-tetrahydro-pyrazolo-[1,2-d][1,4,5]oxadiazepine-7,9-dione
CAS number:	243973-19-5

Animal	Commodity	Source
Bovine blood	Body fluid	Local supermarket

Study Design and Methods

Test facility: Eurofins Agroscience Services Chem SAS
75B, Avenue du Pascalet
30310 Vergèze
France

Study start date: 01 Aug 2022
Study end date: 10 Oct 2022
Analytical phase dates: 04 Aug 2022-12 Aug 2022

Homogenised sub-samples of each test commodity (10 mL) were fortified with standard solutions of pinoxaden in solvent/mixacetonitrile. Five samples of each matrix were fortified at the limit of quantification (LOQ; 0.01 mg/L) and five at 10x LOQ level (0.1 mg/L). Matrices used were bovine blood. The fortified samples were analysed alongside untreated control samples.

Principle of the method

Samples of bovine blood were extracted by homogenisation 5 min with 10 mL acetonitrile. 1 mL of 5N sulphuric acid and 1 tube containing 4g of magnesium sulfate an 1g of sodium chloride were added. The samples were shaken by hand and centrifuged for 5 minutes at 4700 rpm at 4°C. 1 mL of supernatant was mixed with 9 mL of methanol, 0.5% of formic acid (1/3, v/v). Samples were mixed by vortex before Pinoxaden (NOA407854) is determined by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (*m/z* 315-270) and the confirmatory transition (*m/z* 315-172).

HPLC-MS/MS Conditions

HPLC system:	Shimadzu
Pumps:	HPLC LC20ADXR, Shimadzu
Detector:	Applied Biosystems API 5500 triple quadrupole
Autosampler:	HPLC SIL20ACXR
Column	Zorbax SB-Phenyl (50 x 2.1 mm column, 1.8 µm, Art. No. 859700-912)

Mobile phase: A: Water + 0.05 % (v/v) acetic acid
B: Methanol + 0.05 % (v/v) acetic acid

Time (min)	%A	%B
0.0	70	30
8.0	30	70
8.1	10	90
9.5	10	90
9.6	70	30
13.0	70	30

Flow rate: 0.3 ml/min
Column oven temperature 40°C
Injection volume: 2 µL
Retention time: NOA407854: approx. 5.2 min

Detector API 55000

Ionisation mode	ESI
Source polarity:	Positive
Curtain gas (CUR):	20 (arbitrary units)
Gas 1 (GSI):	40 (arbitrary units)
Gas 2 (GSI):	40 (arbitrary units)
Temperature (TEM):	700°C
Ionspray voltage (IS):	-4500V
Collision gas setting (CAD):	9
Entrance potential (EP):	-10 V
Dwell time	100 msec
Resolution Q1 and Q2	unit

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	CE (V)	DP (V)	CXP (V)	Fragment ions (m/z)	
NOA407854 (M2)	315.0	-95	-10	-35 -50	270.0 172.0	Quantification Confirmation

CE: Collision energy; CXP: Collision cell exit potential; DP: Declustering Potential

Results

Recoveries of Pinoxaden (NOA407854) obtained from bovine blood at each fortification level using method QuEChERS are presented in the table below. Other validation parameters of the method are presented in the following table.

Table A 75: Recovery results from method validation of NOA407854 using the analytical method QuEChERS in bovine blood.

Matrix	Analyte	Fortification level (mg/L)	Individual recoveries (%)	Range of recoveries (%) (n = x)	Mean recovery (%)	RSD (%) Comments
Bovine Blood	NOA407854	Mass transition m/z = 315 → 270 (quantification)				
		0.01	92, 104, 93, 95, 99	92 – 104 (n = 5)	97	5
		0.1	90, 92, 90, 80, 84	80 – 92 (n = 5)	87	6
		Overall		80 – 104 (n = 10)	92	7
		Mass transition m/z = 315 → 172 (confirmation)				
		0.01	93, 105, 92, 96, 101	92 - 105 (n=5)	97	6
		0.1	90, 93, 92, 81, 84	81 – 93 (n=5)	88	6
		Overall		81-105 (n=10)	93	8

Table A 76: Characteristics of the data collection/enforcement analytical method used for the quantification of chemical residues in bovine blood.

Analyte	Pinoxaden (NOA407854)
Equipment/ Chromatographic method	Analysis with an HPLC (Shimadzu) coupled to a tandem mass spectrometer (API 5500, Sciex) with electrospray nebuliser.
Accuracy/ Precision (repeatability)	For the LOQ fortification level (0.01 mg/L) and 10xLOQ fortification level (0.1 mg/L), acceptable mean recoveries in the range of 70 - 120% with a relative standard deviation (RSD) of ≤ 20% were found for Pinoxaden (NOA407854)
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance, no further confirmatory technique is required. No peaks in controls above 30% of LOQ.
Confirmatory method	No separate method needed.
Assessment of matrix effects is presented	Matrix effects of bovine blood matrix were insignificant (≤20% suppression/enhancement), nevertheless matrix matched standards were used for quantification.
Calibration/Linearity	Calibration performed with 7 matrix matched single standard levels
	A working calibration range of 0.2 ng/mL to 20 ng/mL (equivalent to a range from 0.002 mg/L to 0.2 mg/L) was used. The lower margin of the linearity was 20 % of the LOQ, and the upper margin was at least 20 % above the highest concentration in the final extracts
	The detector response was linear for bovine blood and all transitions. **Bovine Blood** Quantification : y= 191298x – 808 8(r^2 = 0.9983) Confirmation: y = 253558x – 13153 (r = 0.9979)
Limit of quantification (LOQ)	Limit of quantification representing the lowest validated level with acceptable recovery and precision, LOQ = 0.01 mg/L
Limit of detection (LOD)	LOD = 0.002 mg/L (20 % of the LOQ, defined as lowest calibration standard)
Extract Stability	8 days
Standard Solution Stability	36 days

Conclusion

Method QuEChERS has been successfully validated for the determination of residues of Pinoxaden (NOA407854) in bovine blood with a limit of quantification (LOQ) of 0.01 mg/L.

(Bejan, I., 2022)